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NEW ASPERGILLI FROM SOIL

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(WITH 6 FIGURES)

During the past three years soils from many areas in this country and abroad have been examined at the Northern Regional Research Laboratory for the purpose of securing from natural sources molds important to the research of the Fermentation Division. In the course of this work five species of *Aspergillus* believed to be new have been isolated. All of these were obtained from southern or tropical soils, and hence can be considered as components of the microfloras of habitats characterized by a comparatively high temperature during all or part of the year.

Two types of cultures were made in the examination of these soils:

1. DILUTION CULTURES: Samples of soils were diluted with approximately 10 volumes of sterile water and shaken vigorously for a period of 10 to 15 minutes. Serial dilution in steps of 1 : 10 were then made from the resulting suspensions and plated. Dilutions employed ranged from 1–1,000 to 1–1,000,000, depending upon the source and type of the sample. For each sample three dilutions were generally plated, and as a rule, two media were employed; namely Czapek-Dox agar (**5**) and an acid-dextrose-

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nitrate agar employed and recommended by Smith and Humfeld (**4**) for the isolation of fungi from soil. Dilution plates were incubated at room temperature and desired forms were isolated as they appeared and sporulated. Plates of this type are valuable since they present some quantitative measure of the total fungus flora and of its constituent members as well. When colonies are reasonably well separated in such plates, they generally develop in a characteristic manner and can be isolated directly by means of a low-power binocular or a good-quality hand lens.

2. STREAK CULTURES: Plates of hay infusion agar of the composition previously employed by Raper (**1**) for the isolation of slime molds were streaked with from one to three loopfuls of the initial 1 : 10 suspension. These plates were likewise incubated at room temperature and were examined with a low-power binocular at 7 to 10 days. No molds grow luxuriantly upon this medium, but a tremendous number and variety of forms can make a very limited development. Furthermore, most forms sporulate well and generally appear fairly characteristic, although commonly of smaller dimensions than upon richer substrata. On the whole, hay infusion agar constitutes a very satisfactory medium for the isolation of molds from natural sources, and one can regularly expect to find some forms in these cultures which do not occur, or cannot be recognized, upon the dilution plates described above.

All of the species currently described have been under observation for a period of at least one year and some for approximately two years. They have been grown upon a wide variety of culture media including Czapek's solution agar and various modifications of the same, and malt extract, potato dextrose, and hay infusion agars. A wide range of incubation temperatures have been employed. Since environmental conditions substantially affect their appearance, these factors will be given somewhat greater emphasis than is generally necessary in describing species of *Aspergillus*.

Aspergillus janus sp. nov.

Coloniae cum mutatione substrati temperaturaeque colore et textura multo variae; capitulis conidicis generum duorum, aut ambobus aut unis aut alteris praesentibus: (1) capitulis magnis albis in conidiophoris longis, vesiculis clava-

tis praeditis, (2) parvis, atro-viridibus in conidiophoris brevibus vesiculis ovatis praeditis oriundis. Capitula alba laxa catenulis conidicis radiantibus et divergentibus 150–200 μ longis composita; conidiophoris longis, tenuibus, plerumque 2–3.5 mm. longis, 8–10.5 μ crassis, levibus, hyalinis; vesiculis clavatis membrana tenui praeditis, 45–60 × 15–18 μ, totis sterigmatibus tectis; sterigmatibus in seriebus duabus, primariis 7–10 × 3.5–4.5 μ, secundariis 6–8 × 2.5–3 μ, conidiis levibus, hyalinis, globosis usque subglobosis, plerumque 2–2.5 μ. Capitula viridia compacta, juvenilia radiantia, matura columnaria, saepe in columnas duas divergentes fissentia, primum cyanea usque cyaneoviridia, vetusta atro-olivaceo-grisea, 60–75 μ in diam. 200–300 μ longa; conidiophoris erectis, 300–400 × 6.5–8 μ, levibus, hyalinis; vesiculis membrana tenui praeditis, plerumque ovoideis et saepe elongatis; totis fertilibus, 20–30 × 12–18 μ; sterigmatibus in seriebus duabus, primariis 7–10 × 4–4.5 μ, secundariis 6–8.5 × 2–2.8 μ; conidiis in massa atro-viridibus, spinulosis, globosis, plerumque 3.2–4.0 μ; cellulis "hülle" crasse tunicatis, globosis usque subglobosis vel elongatus, interdum curvatis et saepe lobatis. In culturis e solo, Panama.

Species characterized by conidial heads of two distinct types, (1) large white heads borne upon long conidiophores terminating in strongly clavate vesicles and (2) smaller, dark green heads borne upon short conidiophores with typically ovate vesicles.

Colonies varying greatly in color and in texture depending upon the substratum and the temperature of incubation. Upon Czapek's solution agar (FIG. 1A) at 24°C., colonies spreading irregularly, after two weeks usually consisting of a central floccose mass 1 to 2 mm. deep, pale yellow-buff in color, bearing few to abundant fruiting structures surrounded by an irregular zone of crowded fructifications with dark green heads occurring in a dense stand adjacent to the substratum and with numerous long-stalked white heads projecting above this layer, hülle cells commonly present in scattered to abundant irregular masses, light yellow in color; reverse in dull yellow to light brown shades. When incubated at 20° C. colonies more restricted, less floccose and consisting almost exclusively of a dense stand of long-stalked white heads, with small green heads absent or developing only in age and arising from trailing aerial hyphae entwined among the white fruiting structures. When incubated at 30–32° C. colonies close-textured, predominantly green but with central area commonly showing irregular patches of massed hülle cells, buff to dull yellow in color. Conidial heads abundant and consistently dark green in color. Reverse in dull brown shades.

White conidial heads loose in texture, consisting of radiating and divergent chains of conidia, commonly 150 to 200 μ in diameter, occasionally larger (FIG. 1C). Conidiophores long, thin, mostly 2 to 3.5 mm. in length by 8 to 10.5 μ in diameter, occasionally larger, erect, essentially uniform in diameter throughout but often marked by numerous and irregularly spaced constric-

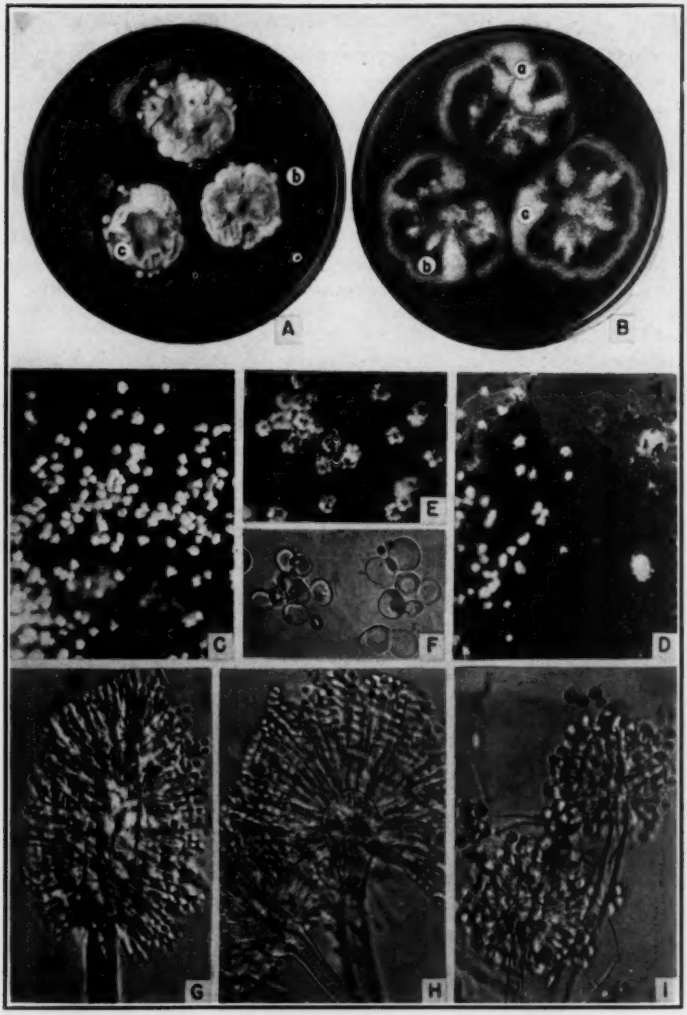

FIG. 1. *Aspergillus janus;* NRRL No. 1787. A and B, colonies on Czapek's solution agar and malt agar, respectively, showing *a*, scattered white conidial heads, *b*, crowded green heads, and *c*, massed hülle cells, two weeks old, incubation at 24° C., × ½. C, characteristic white heads borne on long thin stalks, × 7.5. D, massed green heads in dense stand adjacent to the substratum, with white heads projecting from the left, and with localized develop-

tions, walls smooth, colorless, approximately 1 to 1.4 μ in thickness. Vesicles thin-walled, clavate (FIG. 1G), mostly 45 to 60 μ in length by 15 to 18 μ in width with individual structures larger or smaller, entire surface loosely covered by sterigmata as a rule, but often showing barren areas which may occupy any part of the sterigmatic surface. Sterigmata in two series, primaries 7 to 10 μ by 3.5 to 4.5 μ; secondaries 6 to 8 μ by 2.5 to 3 μ (FIG. 1G). Conidia smooth, colorless, globose to subglobose, mostly 2 to 2.5 μ with maximum about 2.8 μ.

Green conidial heads compact, radiate when young, becoming columnar in age, and often spreading into two divergent columns (FIG. 1D). Heads at first in blue to blue-green shades near dark gobelin blue (Ridgway, Pl. XXXIV), becoming dark olive gray (Ridgway, Pl. LI) in age, in size commonly ranging from 60 to 75 μ in diameter by 200 to 300 μ in length. Conidiophores erect, commonly 300 to 400 μ in length by 6.5 to 8 μ in diameter, of uniform thickness throughout, walls smooth, colorless or very faintly green, approximately 1 to 2 μ thick, enlarging rather abruptly into an elongate vesicle. Vesicle thin-walled, variable in form and dimensions, but commonly ovoid and often conspicuously elongate, typically fertile over the entire area, ranging in size from 20 to 30 μ in length by 12 to 18 μ in diameter (FIG. 1I). Sterigmata in two series, rather loosely arranged, primaries 7 to 10 μ by 4 to 4.5 μ; secondaries 6 to 8.5 μ by 2 to 2.8 μ. Conidia dark green in mass, conspicuously spinulose, globose, mostly 3.2 to 4.0 μ, occasionally larger or smaller.

Conidial heads of mixed character containing both white and green spores often encountered, usually borne upon long conidiophores approaching and often equalling in length those of white heads, vesicles more or less clavate, sterigmata at first bearing colorless smooth-walled conidia, but subsequently bearing dark green spinulose conidia (FIG. 1E and H).

At temperatures of 24° C. and above, thick-walled hülle cells abundant, irregular in form, commonly globose to subglobose, not infrequently elongate, commonly more or less curved and often lobed (FIG. 1F).

Colonies upon malt extract agar growing luxuriantly (FIG. 1B), generally loose in texture with aerial mycelium becoming promi-

ment of hülle cells in two limited areas at right, × 7.5. E, heads of mixed character with older (outer) conidia white and younger (inner) conidia green, × 24. F, hülle cells, × 350. G, typical white head showing clavate vesicle, double sterigmata and small, smooth conidia, × 450. H, head of mixed character, at this stage (early) producing small white, smooth conidia, × 450. I, typical green head, showing small, nearly globose vesicle, double sterigmata and larger, echinulate, green conidia, × 450.

nent in age, conidial heads normally more abundant than upon Czapek's agar, the proportion of white to green heads varying with the temperature of incubation.

Colonies upon hay infusion agar spreading broadly, consisting of a thin submerged mycelium from which develop erect, white and green conidial structures, the relative proportion of these types being dependent upon the temperature of incubation; since comparatively meager growth occurs upon this medium, and since there is a minimum of aerial vegetative hyphae, it constitutes a very favorable substratum upon which to observe the formation of the contrasting fruiting structures characteristic of the species.

The binomial *Aspergillus janus* is applied to this species because of the contrasting types of conidial heads produced—literally it is a "two-faced" mold.

Type culture NRRL No. *1787* was isolated in February 1942, from Panama soil collected during the summer of 1941 by John T. Bonner of Harvard University. Three additional isolations by members of the Northern Regional Research Laboratory staff have since been made from Panama soils subsequently collected by Mr. Benjamin A. Coghill. It is believed that this species represents a normal component of the microflora of Panama. Additional evidence in support of this view is furnished by the fact that in 1925 Professor Roland Thaxter sent to Thom under the label "white Panama *Aspergillus*" a representative of this species. The form was never described by Thaxter, and viable cultures of it were lost from our collection some time prior to 1930. As the correspondence of the time is remembered, Thaxter was plagued by the presence of a small green mold which repeatedly appeared in his cultures as a "contaminant." Fortunately, the original tube received from Thaxter has been preserved, and re-examination of this culture leaves no question but that he was dealing with a strain of the species here described, and that the green form which troubled him so much was not, in fact, a contaminant but a different phase of the same fungus.

The presence of a green phase with conidial heads closely approximating *Aspergillus Sydowi* (Bainier) Thom & Church (**5**) definitely allies *A. janus* with this species; and in the absence of any form approximating the white conidial stage, *A. janus* is placed in the *A. versicolor* group adjacent to *A. Sydowi*.

Extensive experimental studies have established conclusively that the white and green spored forms represent different aspects of a single fungus. These studies will be considered in detail in a subsequent report.

Aspergillus janus var. **brevis** var. nov.

A typo differt conidiophoris brevioribus, vesiculis minoribus, et caespitulis fructiferis viridibus et albis separatim in sectoribus radiantibus dispositis. In culturis e solo, Mexico.

The variety differs from the species in a number of particulars, foremost among which are (1) the reduced length of the conidiophores bearing both white and green heads and (2) a consistent tendency for white and green conidial structures to develop in approximately pure stands and to appear as contrasting radial sectors (FIG. 2 A and B).

White conidial heads are of the same general pattern and form as in the species, but are of somewhat smaller dimensions, are borne upon conidiophores generally less than 2 mm. in length by 6 to 8 μ in diameter, and are characterized by elongate but not strongly clavate vesicles measuring 20–25 μ by 14 to 18 μ; conidia are smooth walled, colorless, globose to subglobose, 2.2 to 2.8 μ in diameter. Green conidial heads are compact, globose to somewhat columnar, borne upon conidiophores 75 to 125 μ by 4 to 6 μ with globose to subglobose vesicles measuring 8 to 15 μ by 10 to 18 μ; conidia are dark blue-green, strongly echinulate, and 3.5 to 4.5 μ in diameter.

The vesicles of white heads in the variety *brevis* are of approximately the same size and form as the vesicles of green heads in the species itself, whereas the conidiophores bearing each type of head are approximately one-half the length of those bearing the same type of head in the species. The most striking character distinguishing the variety, however, is the manner in which areas of white and green heads are sharply separated along radial lines.

Conidial heads of mixed character are produced and usually can be found along the frontiers between white and green sectors. The character of the conidial heads produced is strongly influenced by the temperature of incubation, but this response is not so marked or consistent as in the species itself.

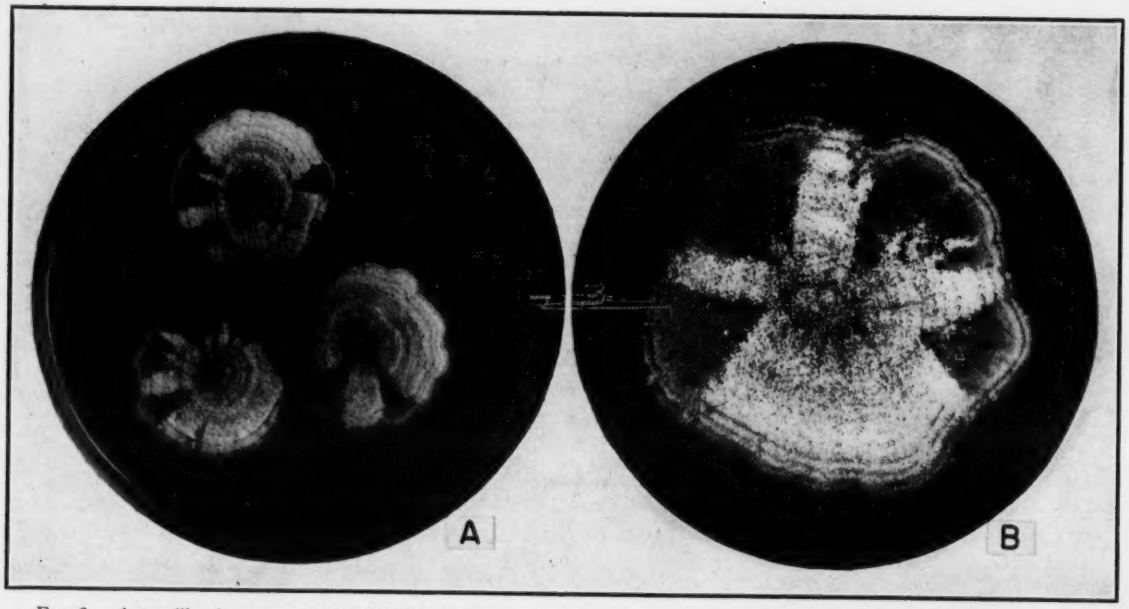

FIG. 2. *Aspergillus janus* var. *brevis;* NRRL No. 1935. A, colonies on malt extract agar, 2 weeks old, incubation at 24° C. Note marked tendency to sector along radial lines. B, single colony enlarged showing greater detail of separation of green and white sporing areas.

Type culture NRRL No. *1935* was isolated in July 1942, from a sample of soil collected in Alameda in southern Mexico, and forwarded to us in June by Mr. William B. Roos.

Aspergillus caespitosus sp. nov.

Coloniae in substratis differentibus multo variantes; in agaro Czapekii tarde crescentes, myceliis submersis, lentis, capitulis conidicis atro-viridibus hemisphaericalibus vel laxe columnaribus, 75–125 μ in diam., cellulis "hülle" abundantibus, cumulos conspicuos maturos rubopurpurascentes formantibus; reverso atro-purpureo; conidiophoris rectis vel subsinuosis, 250–325 $\times$ 5.0–6.5 μ, crasse tunicatis, levibus, alutaceis usque pallide brunneis; vesiculis subelongatis, hemisphaerico superiore sterigmatibus tecto, inferiore sterili, saepe pallido, plerumque 15–20 μ in diam.; sterigmatibus in seriebus duabus, primariis 6.5–8.5 $\times$ 3.5–5.0 μ, secundariis 6.5–8.0 $\times$ 3.0–4.5 μ, typice botuliformibus; conidiis globosis, spinulosis, viridibus, plerumque 3.5–4.5 μ; cellulis "hülle" crasse tunicatis, irregulariter globosis 12–18 μ in diam. vel ovoideis ellipticisve 12–15 $\times$ 25–30 μ, massas compactas subsclerotioideas lentissimas magnitudine varias rubo-purpureas formantibus. In culturis e solo, Arkansas, Arizona, et Texas.

Colonies varying markedly upon different media; upon Czapek's solution agar rather slow growing, attaining a diameter of 6 to 8 cm. in three weeks at room temperature, plane or somewhat furrowed, mycelium largely submerged and extremely tough, tearing with difficulty, producing numerous dark green, hemispherical to loosely columnar heads in central colony areas, characterized particularly by clusters of irregularly ovoid to elliptical, thick-walled hülle cells (FIG. 3 A and B), at first colorless becoming reddish-purple in age; scattered unevenly or arranged in irregularly concentric zones; reverse colorless at first, becoming dark reddish-purple in age, particularly beneath the hülle masses; odor none. Conidial heads dark dull yellow green to empire green (Ridgway, Pl. XXXII), generally hemispherical to loosely columnar (FIG. 3C), mostly 75 to 125 μ in diameter. Conidiophores straight or slightly sinuous (FIG. 3D), mostly 250 to 325 μ in length, occasionally up to 350 μ by 5.0 to 6.5 μ in diameter, of approximately uniform diameter throughout, relatively thick-walled, (1.2 to 1.5 μ in basal portion to 0.8 to 1.0 μ in terminal area), smooth, tan to light brown in color. Vesicle slightly elongate, the upper hemisphere loosely covered by sterigmata (FIG. 3D), the lower half sterile and often lightly colored, mostly 15 to 20 μ in diameter. Sterigmata in two series (FIG. 3D), primaries normally 6.5 to 8.5 μ by 3.5 to 5.0 μ, secondaries 6.5 to 8.0 μ by 3.0 to 4.5 μ, typically bottle-form but commonly much swollen and often quite irregular in form and dimensions. Conidia globose, spinulose, green, mostly 3.5 to 4.5 μ, rarely larger.

Hülle cells very abundant, thick-walled, irregularly globose, ovoid or elliptical (FIG. 3E), ranging from 12 to 18 μ in globose cells to 12 to 15 μ by 25 to 30 μ in the most elongate bodies, forming compacted masses of indefinite size, extremely tough and in age becoming almost sclerotioid, at first colorless but in age charac-

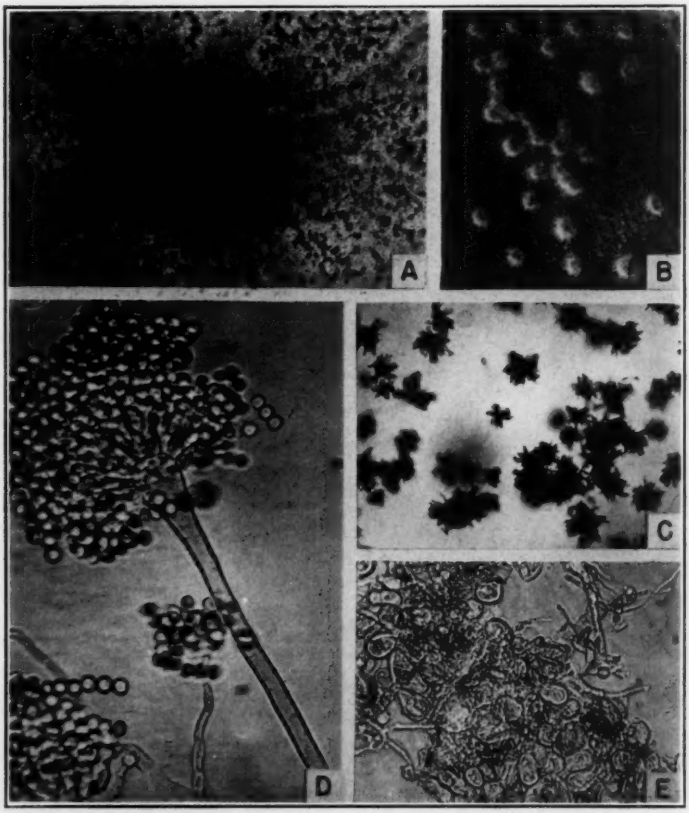

FIG. 3. *Aspergillus caespitosus;* NRRL No. 1929. A, portion of colony on Czapek's solution with 1% liver extract, showing crowded conidial heads in central portion and scattered hülle cell masses in surrounding areas, two weeks old, incubation at room temperature, × 1.8. B, portion of colony enlarged showing hülle cell masses, × 10. C, silhouettes of conidial heads developed on hay infusion agar, × 48. D, typical conidial head showing form of vesicle and arrangement of sterigmata, × 600. E, portion of hülle cell mass showing irregular size and form of component elements, × 265.

terized by an abundant reddish-purple intercellular pigmentation.

Colonies upon malt agar characterized by a dense stand of erect conidiophores bearing hemispherical to radiate or loosely columnar heads of dark green color approximately empire green (Ridgway, Pl. XXXII) and the complete absence of hülle cells; reverse in light brown shades; odor none. Details of morphology as upon Czapek's solution agar.

Colonies upon hay infusion agar (FIG. 3C) like those upon malt except less heavily sporing.

The binomial *Aspergillus caespitosus* is chosen because of the caespitose character of colonies upon Czapek's solution agar.

Strains examined include NRRL No. *1929* isolated from Arkansas soil collected by F. R. Earle at Fayetteville, Arkansas; NRRL No. *1930* isolated from Arizona soil contributed by C. J. King; and strains isolated from Texas soils contributed by various collaborators.

This species is of particular interest because of its apparent transitional position between the *A. nidulans* group and *A. ustus*. In the character of its stalks, its reddish-purple pigmentation, and in the general color and markings of its conidia it retains the characters of *A. nidulans* and closely related species. In the absence of fertile perithecia and ascospores, the predominantly hemispherical shape of its conidial heads, and in the variable and somewhat irregular form of its hülle cells it is suggestive of the *A. ustus* series. While we are convinced of its intermediate position between the *A. nidulans* group and *A. ustus*, we place it with the former since we believe it is most closely allied to this group. It is believed significant that superficially, cultures of *Aspergillus caespitosus* and *Aspergillus variecolor* (Berk. & Br.) Thom & Raper (6) are strikingly similar upon Czapek's solution agar. This similarity is particularly marked when plates are viewed in reverse since an intense pigmentation marks the under surface of perithecia in the latter species and the under surface of older hülle masses in the former.

Aspergillus granulosus sp. nov.

Coloniae in agaro Czapekii planae vel irregulariter rugulosae, floccosae, alutaceae vel obscure brunneae; capitulis conidicis paucis, plurimum caespitosis, pallide cyaneo-viridibus, hemisphaericalibus usque radiantibus, 75–125 μ in diam.; conidiophoris erectis, aseptatis, 350–500 $\times$ 5.5–8.0 μ, membrana

tenui et levi praeditis, ex alutaceo pallide brunneis, saepe infra vesiculas constrictulis; vesiculis ovatis usque ellipticis, membrana tenui praeditis, 12–18 μ in diam., 15–25 μ longis, fragilibus, parte maiore sterigmatibus vestitis; sterigmatibus in seriebus duabus, primariis 3.5–5.0 × 3.0–4.0 μ, secundariis 4.0–5.5 × 3.0–3.5 μ; conidiis globosis, pallide viridulis, tenuissime echinulatis, 4.8–5.5 μ in diam.; cellulis "hülle" abundantibus, irregulariter globosis, ovoideis vel aliquantus elongatis, 12–30 μ in diam., crasse tunicatis, in cumulos parvos, conspicuos margine coloniae aggregatis et culturas granulosas efficientibus. In culturis e solo, Arkansas, Texas, Arizona, et Costa Rica.

Colonies upon Czapek's solution agar growing well, attaining a diameter of 8 to 10 cm. in two to three weeks at room temperature, plane or irregularly furrowed, predominantly floccose, uneven in texture, buff to dull brown in color from felted sterile mycelia; conidial heads few in number and generally arising from the substratum direct, less often from aerial hyphae, commonly appearing in clusters, pale blue-green in color; colonies characterized particularly by abundant small, colorless clusters of irregularly globose ovoid, or elliptical thick-walled hülle cells which superficially suggest perithecial initials and which in mass give to the colony a semi-granular appearance (FIG. 4 A and B); reverse in shades of dull yellow and brown; slight mushroom odor. Conidial heads few in number, commonly clustered in small groups, most abundant at colony margin, sometimes occurring on tufts of aerial hyphae, hemispherical to radiate, 75 to 125 μ in diameter, very loose, consisting of comparatively few divergent spore chains (FIG. 4C), approximately pale niagara green in color (Ridgway, Plate XXXIII). Conidiophores erect, straight, non-septate, mostly 350 to 500 μ in length, 5.5 to 8.0 μ in diameter, approximately uniform in width throughout, thin-walled, smooth, tan to light brown in color, often slightly constricted just beneath the vesicle. Vesicle ovate to elliptical (FIG. 4D), 12 to 18 μ in diameter by 15 to 25 μ in length, thin-walled and easily broken, largely covered by sterigmata. Sterigmata in two series, both comparatively short and stout; primaries 3.5 to 5.0 μ by 3.0 to 4.0 μ; secondaries 4.0 to 5.5 μ by 3.0 to 3.5 μ, commonly bottle-form. Conidia globose, pale green, delicately echinulate. Mostly 4.8 to 5.5 μ in diameter, rarely larger. Hülle cells abundant, irregularly globose, ovoid or somewhat elongate (FIG. 4F), commonly 12 to 30 μ in long axis, walls heavy, 4 to 5 μ in thickness, borne primarily in small, colorless clusters which are quite conspicuous at colony margins and lend to them a characteristic granular appearance (FIG. 4 B and E).

Colonies upon malt extract agar showing an accentuation of hülle cell development and a reduction in conidial heads. Otherwise duplicating the cultural picture presented upon Czapek's solution agar.

Colonies upon hay infusion agar thin but broadly spreading, characterized by scattered clusters of hülle cells and erect conidial fructifications giving to the culture a sparse granular appearance.

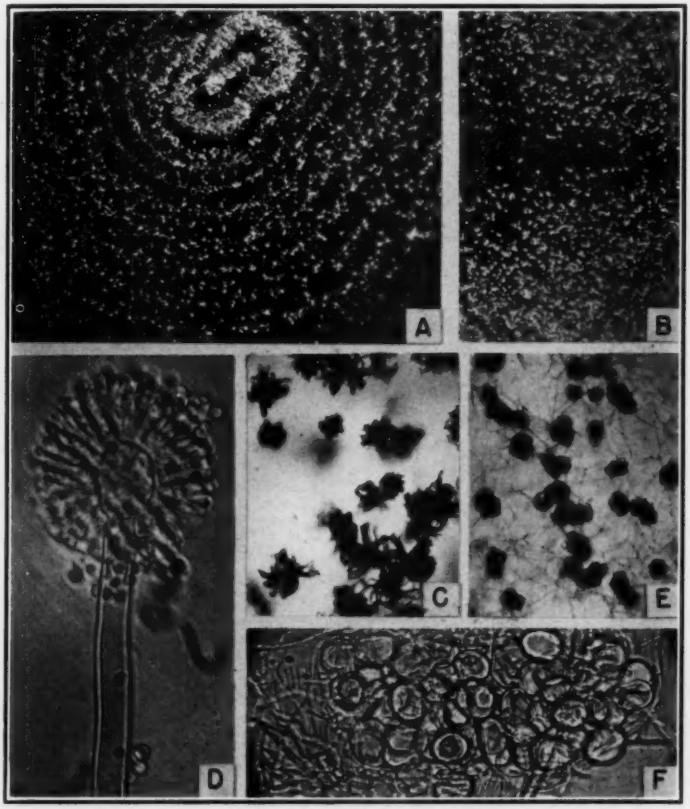

FIG. 4. *Aspergillus granulosus;* NRRL No. 1932. A, portion of colony on hay infusion agar showing clusters of conidial structures and small masses of hülle cells, two weeks old, incubation at room temperature, × 1.5. B, portion of marginal area of two colonies on malt extract agar showing characteristic granular appearance resulting from numerous small clusters of hülle cells, × 1.5. C, silhouettes of conidial heads, × 48. D, conidial head showing slightly elongate vesicle, sterigmata in two series, and constriction in conidiophore just beneath the vesicle, × 750. E, small clusters, or "granules," of hülle cells, × 48. F, double cluster of hülle cells, much enlarged, × 265.

The binomial *Aspergillus granulosus* is chosen because of the conspicuous granular appearance of the colonies produced upon most laboratory substrata.

Type culture NRRL No. *1932* was isolated in September 1942, from a sample of soil collected in Fayetteville, Arkansas, and contributed by Mr. F. R. Earle. Additional strains have been isolated from soils collected in Texas, Arizona, and Costa Rica. It is believed common in soils characterized by a warm temperature during part or all of the year.

Different strains vary materially in the number of conidial heads produced upon common laboratory media such as Czapek's solution and malt extract agars, ranging from abundant heads in some to only widely scattered heads in others. All fruit reasonably well, however, upon hay infusion agar, the medium upon which all original isolations were made.

The brown color of the conidiophores, the presence of ovoid to somewhat irregular hülle cells, and the green color of its conidia ally this species with *A. ustus*. It differs markedly from the more common representatives of this group, however, in the lighter and persistently green color of its conidia, the small clusters rather than irregular masses of hülle cells, and in possessing somewhat more elongate vesicles. In this latter character it suggests *Aspergillus flavipes* but is in turn excluded from this group by the green color of its spores.

Aspergillus panamensis sp. nov.

Coloniae in agaro Czapekii pertenues, paene totae submersae, fructificationes conidicas late dispersas capitulis radiantibus pallide brunneis gerentes, in agaro maltoso luxurianter fructificantes, rubro-brunneae; capitulis radiantibus, pallide brunneis, typice globosis, 250–450 μ in diam., ex avellaneo rubro-brunneis; conidiophoris rectis, 600–900 × 9–12 μ, membrana comparative crassa praeditis, levibus; vesiculis hyalinis, membrana tenui praeditis, globosis vel subelongatis, 25–30 μ in diam., totis fertilibus; sterigmatibus in seriebus duabus, dense compactis, primariis 5.5–6.5 × 2.4–2.8 μ, secundariis 5–6 × 1.5–2 μ, conidiis in massa pallide flavo-brunneis, globosis usque subglobosis, levibus, 2.2–2.6 μ in diam. In culturis e solo, Panama.

Colonies upon Czapek's solution agar after two weeks at room temperature very thin, consisting of a sparse and transparent growth of vegetative hyphae, almost wholly submerged, bearing widely scattered, erect conidial structures with radiate heads (FIG. 5A), light brown in color. Colonies upon malt extract agar at

room temperature growing well and fruiting luxuriantly (FIG. 5B), reddish-brown in color, consisting of a dense, predominantly red basal mycelium from which develops massed conidial structures, often in broken or continuous concentric zones; fertile

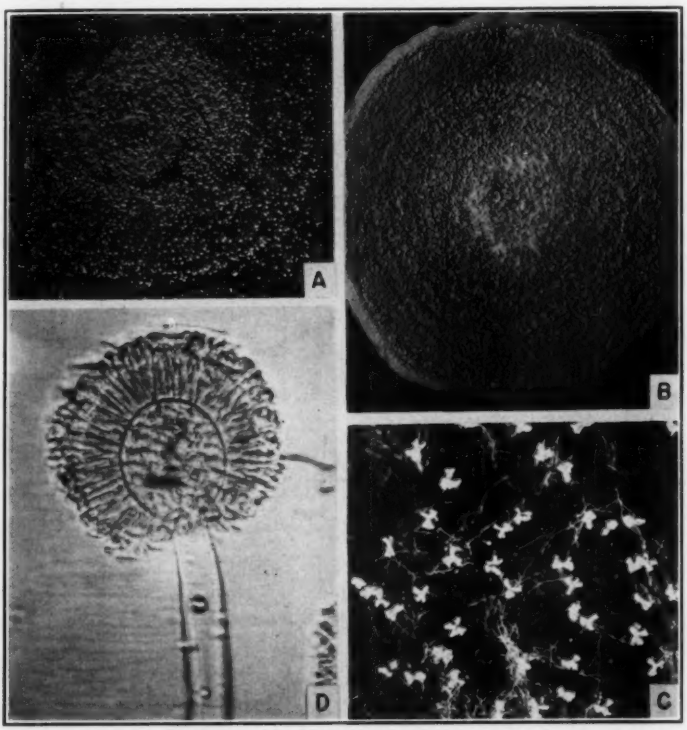

FIG. 5. *Aspergillus panamensis;* NRRL No. 1785. A, portion of a single colony on Czapek's solution agar with 1% liver extract showing limited growth and scattered conidial heads, 2 weeks old, incubation at room temperature, × 1.5. B, luxuriant growth and heavily sporing colony on malt extract agar, age and incubation as in preceding, × 1.5. C, conidial heads showing characteristic forms when mature, hay infusion agar, × 8. D, single conidial head showing almost globose vesicle, closely packed, double sterigmata, × 500.

conidiophores bearing globose to radiate heads, light brown in color, near wood brown of Ridgway (Pl. XL), these, together with red-colored sterile structures and aerial hyphae give the colony its characteristic appearance and color; in age, colonies tending to

develop a loose floccose overgrowth, more or less obscuring the abundant conidial heads; reverse dull brown; odor none. Colonies upon hay infusion agar spreading broadly, thin, largely submerged, giving rise to scattered, erect conidial structures commonly arranged in concentric zones; heads radiate, dull brown in color. Conidial structures arising directly from the substratum, scattered or abundant depending upon the culture medium employed. Heads typically globose, in age characterized by loosely radiating chains of conidia, or by few to several roughly columnar masses, (FIG. 5C), variable in size, commonly ranging from 250 to 450 μ in diameter, occasionally up to 500 μ, varying in color from avellaneous to wood brown (Ridgway, Pl. XL) to Saccardo's umber (Ridgway, Pl. XXIX). Conidiophores straight, mostly 600 to 900 μ in length by 9 to 12 μ in diameter, occasionally larger; walls smooth, comparatively heavy, ranging from 3 to 3.5 μ thick in the basal area to 1.5 to 2 μ in the terminal area, approximately uniform in diameter throughout except for a limited reduction immediately beneath the vesicle. Vesicle colorless, comparatively thin-walled, globose or slightly elongate (FIG. 5D), mostly 25 to 30 μ in diameter, fertile over the entire area. Sterigmata in two series, closely packed, primaries 5.5 to 6.5 μ by 2.4 to 2.8 μ, secondaries 5 to 6 μ by 1.5 to 2 μ. Conidia light yellowish-brown in mass, globose to subglobose, smooth-walled, mostly 2.2 to 2.6 μ in diameter, occasionally 2.8 μ.

The binomial *Aspergillus panamensis* is chosen because of the place of species origin.

Type culture NRRL No. *1785* was isolated in January 1942, from Panama soil collected in the summer of 1941 by Mr. John T. Bonner. A second culture, NRRL No. *1786*, differs from the above strain in minor details but clearly belongs with it. This was isolated from a different sample of Panama soil collected by Bonner.

The species is believed to represent a member of the *Aspergillus wentii* group with possible close affinity to *Aspergillus niger* mut. *cinnamomeus*[3] (syn. *Aspergillus cinnamomeus* Schiemann) and *Aspergillus niger* mut. *Schiemanni*[4] (syn. *A. fuscus* Schiemann). While it bears a certain resemblance to these forms in the com-

[3] Nomenclature according to Thom and Raper—"Manual of The Aspergilli," now in preparation.

[4] Nomenclature according to Thom and Raper—"Manual of The Aspergilli," now in preparation.

paratively light color of its conidial heads and in the smallness and general character of its conidia, it differs from these forms in three very striking particulars. (1) It characteristically develops an extensive red-colored aerial mycelium upon media such as malt extract agar where it attains its maximum growth; (2) it grows very sparsely upon Czapek's solution agar, upon which the above noted forms grow luxuriantly; and (3) it possesses very small primary sterigmata, measuring 5.5 to 6.5 μ by 2.4 to 2.8 μ in contrast to 15 to 18 μ by 3 to 5 μ for mut. *cinnamomeus* and 15 to 40 μ by 4 to 6 μ for mut. *Schiemanni*. Whether or not the species actually represents a naturally occurring mutation from *A. niger* can only be guessed. The smallness of its conidia and primary sterigmata would hardly support this hypothesis. In cases where color mutations have been obtained from known cultures, the dimensions of specific structures in such mutations generally agree very closely with those of the same structures in the parent strain; and black *Aspergilli* with the dimensions of *A. panamensis* are rarely, if ever, encountered in nature. The possibility of this representing a mutation is not excluded, but until additional evidence supporting such origin is forthcoming, the writers feel warranted in describing as new this unique form which obviously is able to maintain itself in the soils of Panama.

The luxuriant growth of the fungus upon malt extract agar and its very sparse development upon Czapek's solution agar is strongly suggestive of other cultures which we have found to possess vitamin deficiencies or an inability to utilize nitrate nitrogen. In this species, however, the paucity of growth upon our standard Czapek's agar appears to result from an absence or inadequacy of invertase production since it grows well and fruits quite abundantly upon Czapek's solution containing glucose instead of sucrose. In the presence of other microorganisms capable of reducing sucrose, the fungus grows well and fruits abundantly upon Czapek's solution agar.

A recurring phenomenon typical of this species is the manner in which massed conidiophores develop in broken or continuous concentric zones in the growing colony upon malt extract and other favorable media. Particularly characteristic is the failure of great numbers of these conidiophores to produce fertile heads and the

lateness with which others develop such reproductive structures. While no suggestion of close relationship is intended, these sterile structures bear a certain superficial resemblance to the "spears" characteristic of *Aspergillus unguis* (Emile-Weil & Gaudin) Emnd. Thom & Raper as described by Thom and Raper (6).

Strain NRRL No. *1876*, likewise isolated from Panama soil, differs from the type in producing somewhat more restricted colonies. These are, in addition, less highly colored, due primarily to a reduction in the amount of red colored vegetative hyphae and in the number of aborted conidiophores. It is similar to it, however, in all basic morphological characteristics, and, like it, makes a very sparse growth upon Czapek's solution agar containing sucrose but grows well and fruits luxuriantly upon malt extract agar and Czapek's agar containing dextrose.

Aspergillus sparsus sp. nov.

Coloniae in agaro Czapekii late crescentes, griseo-brunneae, primum submersae dein aeriae, fructificationibus conidicis erectis, late dispersis, viridialutaceis, in agaro ex infusione foeni sparsis, conspicuis, saepe concentrice zonatis, capitulis globosis vel radiantibus vel irregulariter scindentibus, 200–250 μ in diam.; conidiophoris rectis, 1–1.5 mm. longis, 10–12 μ latis, conspicue echinulatis, saepe infra vesiculas abrupte attenuatis; vesiculis membrana comparative tenui praeditis, globosis, plerumque 40–50 μ in diam., totis fertilibus; sterigmatibus in seriebus duabus, dense confertis, primariis vulgo 8–10 $\times$ 3–5 μ, secundariis 6–8 $\times$ 2.5–3.5 μ; conidiis in massa pallide flavidulis, subglobosis usque ellipticis, minute asperulis, plerumque 3–3.5 μ. In culturis e solo, Honduras et Texas.

Colonies upon Czapek's solution agar at room temperature spreading broadly, dull grayish-brown in color, at first largely submerged but later developing limited aerial growth, giving rise to widely scattered erect conidial structures characterized by dull greenish-tan heads that do not affect the color of the colony as a whole; reverse in brown shades; odor none. Colonies upon Czapek's solution agar with 20 per cent sugar slightly heavier sporing (FIG. 6A). Conidial structures erect, arising from a submerged mycelium; heads typically globose radiate to splitting irregularly in age (FIG. 6B), mostly 200 to 250 μ in diameter, occasionally as much as 500 μ, pale olive-buff to olive-buff (Ridgway, Pl. XL) upon Czapek's and hay infusion agars to pale olivine or olivine (Ridgway, Pl. XXXII) upon malt extract agar. Conidiophores straight, mostly 1 to 1½ mm. in length by 10 to 12 μ in diameter, approximately uniform in diameter throughout, walls 1.2 to 1.5 μ in thickness, conspicuously roughened, typically

arising from foot cells enmeshed in a network of "feeder" hyphae, often tapering abruptly in the region immediately beneath the vesicles (FIG. 6 C and D). Vesicles comparatively thin-walled, globose, mostly 40 to 50 μ in diameter, occasionally larger or smaller, bearing sterigmata over the entire surface (FIG. 6C). Sterigmata in two series, crowded, comparatively short and stout,

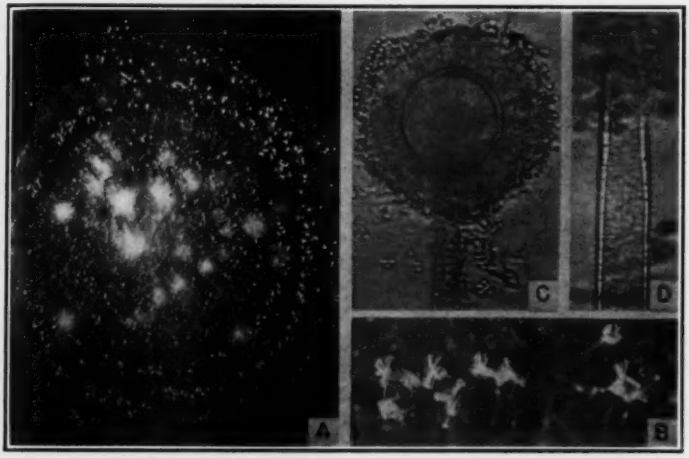

FIG. 6. *Aspergillus sparsus;* NRRL No. 1933. A, single colony on Czapek's solution agar with 20% sucrose showing limited growth and scattered conidial heads, 2 weeks old, incubation at room temperature, × 1.5. B, conidial heads, showing characteristic splitting, and the generally loose arrangement of conidial chains, × 8. C, conidial head showing globose vesicle, closely packed, double sterigmata and terminal portion of roughened conidiophore, × 400. D, further enlargement of conidiophore showing characteristic echinulations, × 650.

primaries commonly 8 to 10 μ by 3 to 5 μ, secondaries 6 to 8 μ by 2.5 to 3.5 μ. Conidia pale yellowish in mass, individually showing slight coloration, subglobose to slightly elliptical, very finely roughened, mostly 3 to 3.5 μ in long axis.

Colonies on hay infusion agar spreading broadly, almost wholly submerged, giving rise to scattered but conspicuous conidial structures, often in definite concentric zones; heads globose, radiate, in olive-buff shades. Colonies upon malt extract agar spreading irregularly, floccose, 1 to 2 mm. deep, cream-buff in color; conidial structures very few in number, heads globose, radiate, dull yellow-green in color.

The binomial *Aspergillus sparsus* is chosen for this species because of its characteristic sparse production of conidial structures upon all substrata employed.

Type culture NRRL No. *1933* was isolated in February 1943, from soil collected in La Lima, Honduras, by Dr. L. A. Underkofler in December 1942. A second strain, NRRL No. *1937*, which duplicates the type almost exactly was subsequently isolated from soil collected in Bixar County, Texas, by Sister Mary Clare of Our Lady of the Lake College, San Antonio, Texas.

The correct position of this species within the genus *Aspergillus* is open to question. The presence of a colored, coarsely roughened stalk indicates close relationship with *Aspergillus ochraceus*. This is likewise supported by the globose vesicle and head, although these characters are typical of other groups as well. The scarcity of fruiting structures upon all media, and more particularly the greenish tint of the spore masses, however, tend to set it apart from the common representatives of this great group. The general habit of the colonies, together with the paucity of conidial structures, is strongly suggestive of *Aspergillus alliaceus*, but this latter species does not show any green color in its conidial heads; it possesses smooth, colorless conidiophores; and upon ordinary culture media regularly produces an abundance of black sclerotia which very often dominates and characterizes the culture. In the color and character of its conidial heads it is somewhat suggestive of George Smith's new species, *A. avenaceus* (**3**) but it differs from this fungus as it does from *A. alliaceus* in possessing rough conidiophores and in its failure to produce sclerotia. Until additional related forms are isolated, we believe it best to consider this species as a member of the *Aspergillus ochraceus* group, realizing that it does not entirely fit this placement as the group has hitherto been considered.

SUMMARY

Five species and one variety of *Aspergillus* are described as new. All of these forms were isolated from southern or tropical soils and are believed to represent normal components of the microfloras of the areas from which they were obtained.

The new forms include:

(1) *Aspergillus janus*, characterized by conidial structures of two types, namely, white heads with clavate vesicles borne upon

long conidiophores, and blue-green heads with globose to ovate vesicles borne upon short conidiophores. This species is believed closely related to *Aspergillus Sydowi* (Bainier) Thom & Church.

(2) *Aspergillus janus* var. *brevis*, differing from the species in the smaller dimensions of both white and green heads and in the marked tendency for colonies to develop alternate radial sectors of white and green heads in approximately pure stands.

(3) *Aspergillus caespitosus*, a non-ascosporic member of the *A. nidulans* group, characterized by irregular masses of hülle cells and non-columnar heads.

(4) *Aspergillus granulosus*, a member of the *A. ustus* group, characterized by small clusters ("granules") of hülle cells and a limited production of pale blue-green conidial heads.

(5) *Aspergillus panamensis*, a form suggestive of the *A. niger* group but characterized by avellaneous to dull brown conidial heads and an apparent invertase deficiency.

(6) *Aspergillus sparsus*, a member of the *A. ochraceus* group, characterized generally by limited growth and the production of few conidial structures, with conidiophores strongly echinulate and globose heads approximately olive-buff in color.

ACKNOWLEDGMENT

The writers are greatly indebted to Miss Edith K. Cash for preparing the Latin diagnoses, to Mrs. Dorothy F. Alexander for assistance in cultural studies and in the preparation of illustrations, and to our collaborators who collected and sent to us the soils from which these fungi were isolated.

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A STUDY OF THE APPLE ROT FUNGUS PHIALOPHORA MALORUM

L. P. McCOLLOCH

(WITH 4 FIGURES)

In a preliminary note (**9**) the author discussed briefly a rot of stored apples and pointed out that the fungus causing the rot should be properly designated as *Phialophora malorum* (Kidd & Beaum.) McColloch, with *Sporotrichum carpogenum* Ruehle as a synonym. The serious losses occasioned by this fungus have warranted further studies and an account will be given here in greater detail of its physiology, morphology and taxonomy.

Phialophora malorum is primarily a saprophyte living in the surface soil and upon the bark and in cankerous woody tissue of apple trees in the orchard. Apples become infected while on the trees and under favorable conditions the fungus develops and causes serious and unpredictable losses in fruit in storage. The majority of lesions occurring on Winesap apples appear to develop around the lenticels, yet it is difficult to determine in many cases whether the point of entrance is through a lenticel or through cuticular cracks. The fungus also enters through insect injuries and mechanical punctures.

SOURCE OF CULTURES

Many cultures were made from decayed areas in stored apples from Virginia over a period of three years and occasional cultures were made from decayed apples grown in Maryland and Washington. In addition others were obtained from cankerous wood of apple trees and surface soil from Virginia during September 1937 and February 1939. The Centraal Bureau voor Schimmelcultures, Baarn, Holland, supplied a culture of Kidd and Beaumont's "*Sporotrichum malorum*" and the Botany Department of Washington State College, one of Ruehle's "*Sporotrichum carpogenum*." A transfer of *Phialophora verrucosa* Medlar was furnished by Dr. Charles Thom and three wood staining species, *Phialophora fasti-*

giata (Lager. & Melin) Conant, *P. brunnescens* (Davidson) Conant, and *P. repens* (Davidson) Conant, were obtained from Ross W. Davidson of the Division of Forest Pathology, Bureau of Plant Industry.

Single-spore cultures were obtained from all of the isolates made by the author as well as from several cultures received from other sources. Sub-cultures were periodically set up from the single spore lines, using spores and bits of mycelium taken from the central part of the old culture. Stock cultures were carried on Thaxter's agar. As a preliminary step all isolates were tested on fruit to determine the extent of pathogenicity and only those found to produce apple decay were included in the general study.

After preliminary studies of the entire group of cultures, ten representatives were selected for more detailed study. The sources of the ten cultures included in the study are listed in table 1.

TABLE 1

SOURCES OF THE 10 STRAINS OF PHIALOPHORA MALORUM INCLUDED IN THE DETAILED STUDY

Serial number of culture	Source of cultures
92	*Sporotrichum malorum* (Kidd & Beaum.) Centraal Bureau voor Schimmelcultures, Baarn, Holland
90	*Sporotrichum carpogenum* Ruehle, Botany Department, State College, Pullman, Washington
128	Isolated from fruit lesion, Winesap apple, from Virginia
132	Isolated from fruit lesion, Winesap apple, from Virginia
158	Isolated from fruit lesion, Winesap apple, from Virginia
159	Isolated from fruit lesion, Winesap apple, from Virginia
163	Isolated from fruit lesion, Winesap apple, from Virginia
172	Isolated from fruit lesion, Winesap apple, from Virginia
186	Isolated from soil beneath Winesap tree, from Virginia
231	Isolated from cankerous area on Winesap tree, from Virginia

CULTURAL STUDIES

The several cultures grew well, but slowly, on most of the nutrient media tried. The extent of aerial development and the color of the mycelial mats were greatly influenced by the nutrients supplied in the artificial media. Development on water agar was scant, but the hyphae developed a smoky color. Heineman's asparagin-peptone agar with washed starch as the carbohydrate

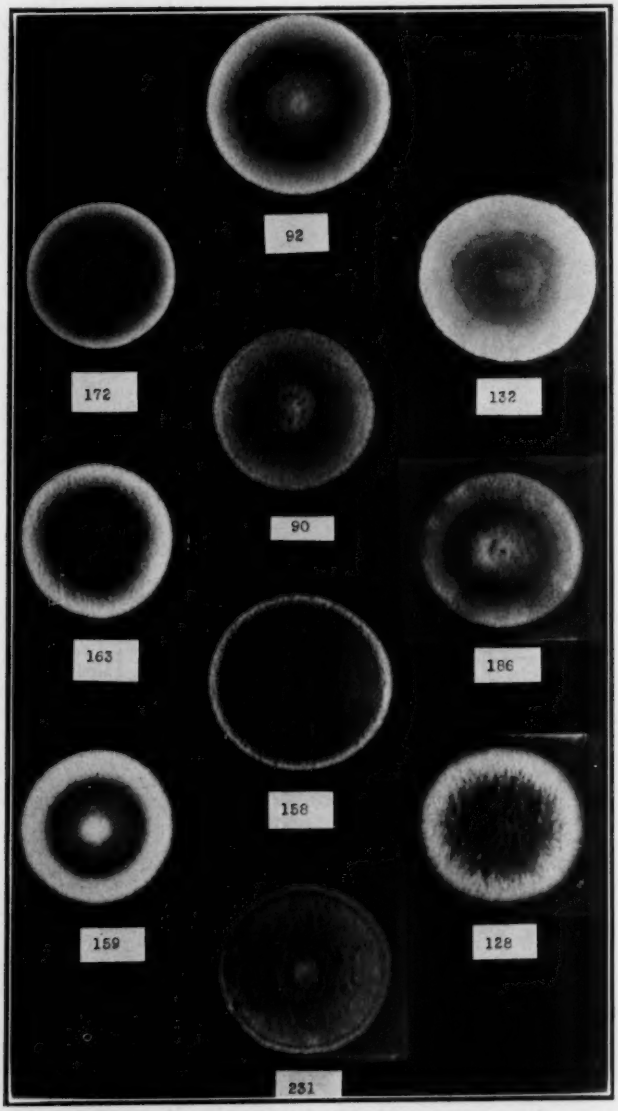

FIG. 1. General appearance of *Phialophora malorum* strains on Thaxter's agar.

source greatly retarded the development of the fungi. The fungus mats were wet, appressed and without aerial hyphae during the first ten days of growth. After eighteen days at room temperature, cultures 90, 92, 159, and 163 had failed to produce dark pigment; cultures 128 and 158 had developed a brown pigment; while 132, 172, 186 and 231 approached the normal degree of color. The rate of growth on Czapek's agar was fairly normal, but the mats were wet and appressed in all except No. 132 which was velvety, and 172 which was woolly instead of having the usual velvety texture. There was a great variation in the ability of these organisms to produce dark pigment on this medium. Cultures 159 and 163 remained a dingy white or yellowish white after 20 days at room temperature. Thaxter's agar, composed of unoxidized potato juice and two per cent dextrose, induced better development than other plant decoctions or synthetic media tested. The cultures responded so well on this medium (FIG. 1) that numerous observations were made on the form, texture, and mass color of the fungus mat, notwithstanding the fact that such a medium cannot be accurately duplicated. The color of the organisms varied on each medium (table 2), but Thaxter's medium with five per cent of agar noticeably unified the coloring, as compared with the same medium with 1.5 per cent of agar.

INFLUENCE OF HYDROGEN-ION CONCENTRATION

A study was made of the ten cultures, using beef infusion broth with hydrogen-ion concentrations from pH 8.35 to pH 2.5. Germination occurred at pH 3.5 after 24 hours, and after a longer period of time at pH 2.9 and pH 2.5. After seven days at room temperature, the extent of growth was noted and pH readings were made to determine the extent to which the hydrogen-ion concentrations had been changed by the fungus development.

The data showed individual differences in the reaction of the fungi at a given concentration but they all decreased the hydrogen-ion concentration from that originally used. Judging from the extent of growth and the change in hydrogen-ion concentration, the best development occurred between pH 4.4 and 6.0 which is in agreement with Gardner's (**3**) spore-germination studies.

TABLE 2

COLOR AND TEXTURE OF 10 STRAINS OF PHIALOPHORA MALORUM ON TWO TYPES OF CULTURE MEDIA

Serial no.	On Thaxter's 2 per cent agar		On 1.5 per cent Difco malt agar	
	Color of mycelial mat according to Ridgway's color standards	Texture	Color of mycelial mat according to Ridgway's color standards	Texture
92	Uniform color—nearest to dark olive gray	Compact woolly	Central area, grayish olive; outer part, mineral gray	Compact woolly
90	Uniform color—between deep olive and dark olive gray	Compact woolly	Pale gull gray to gull gray	Open woolly, surface coarse webby
128	Uniform color—between deep olive and dark olive gray	Spinose	Light olive gray	Spinose, becoming webby
132	Three color bands: center, light grayish olive; mid-portion, nearest smoke gray; margin, pale smoke gray	Dense, fine velvety	Center, light olive gray; remainder, pale drab gray	Dense, fine velvety
158	Uniform color—between deep olive and dark olive gray	Open woolly	Light olive gray to olive gray	Open woolly
159	Uniform color—deep olive gray	Dense woolly	Pale olive gray	Dense, compact woolly
163	Uniform color—light grayish olive	Open woolly	Light grayish olive	Open woolly
172	Two color bands: center, deep grayish olive; outer, between light grayish olive and grayish olive	Dense, fine velvety	Central part, light drab; remainder, light olive gray	Dense, fine velvety
186	Uniform color—nearest to dark grayish olive	Soft woolly	No reading made	Wet, appressed
231	Uniform color—nearest to deep olive	Soft woolly	Light olive gray	Dense, compact woolly

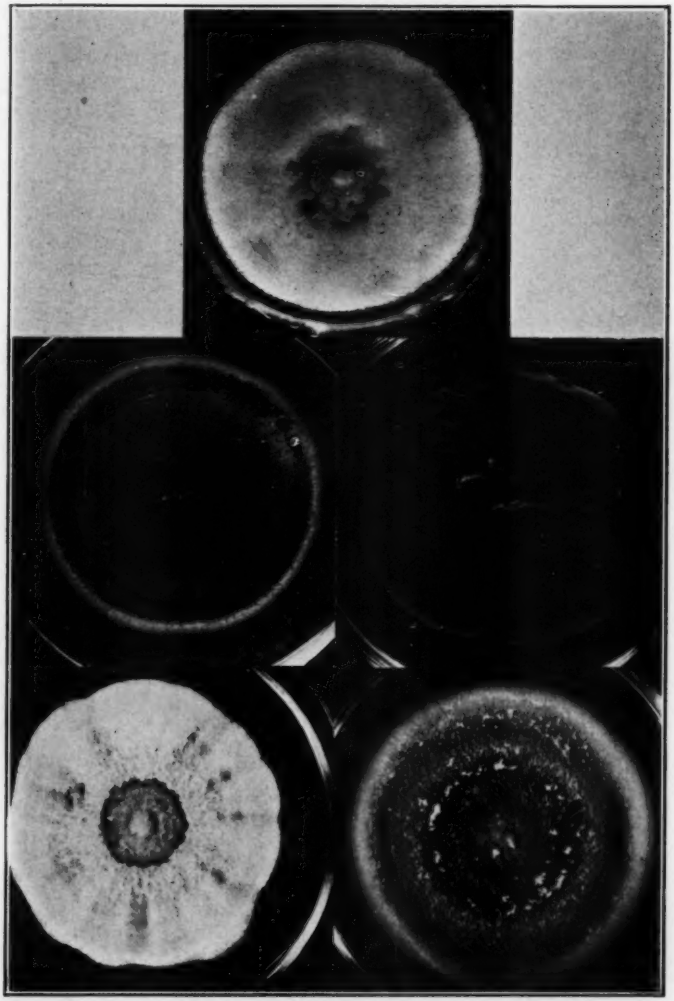

FIG. 2. Culture No. 90 at top center with four of its saltants shown below.

OXIDASE REACTION

The method used in testing the cultures for oxidase production was the same as that described by Davidson (1), using .5 per cent tannic acid with 1.5 per cent Difco malt, and .5 per cent gallic acid with 1.5 per cent Difco malt, each made up with 2 per cent agar. The cultures were incubated at room temperature. The diameter of the fungus colony, and the browned area caused by the oxidation of the acids were measured periodically to show the rate of development.

The fungus reaction to these media varied more in the first four days than after 14 days or longer. Eventually, all cultures gave positive oxidase reaction on tannic acid medium. Likewise all cultures except No. 158 gave positive reactions to gallic acid medium, although there was considerable difference in the rate and intensity of oxidase production.

ACID PRODUCTION

In testing for the production of acid, a synthetic medium using glycerine as the carbohydrate source was adjusted in pH 7.0, and bromcresol purple was added as an indicator. On the eighth and ninth days at room temperature, measurements were made of the

TABLE 3

RATE AND EXTENT OF ACID PRODUCTION BY 10 STRAINS OF PHIALOPHORA MALORUM WHEN GROWN ON A SYNTHETIC MEDIUM CONTAINING BROMCRESOL PURPLE AND ADJUSTED TO pH 7.0

Culture number	After 8 days			After 9 days		
	Diameter in mm.		Difference	Diameter in mm.		Difference
	Fungus colony	Yellow zone		Fungus colony	Yellow zone	
92	23.0	31.5	8.5	26.0	39.5	13.5
90	23.0	24.0	1.0	26.5	28.5	2.0
128	22.0	34.0	12.0	27.0	44.0	17.0
132	20.0	22.0	2.0	22.0	31.0	9.0
158	24.0	34.0	10.0	28.0	45.0	17.0
159	23.0	26.0	3.0	25.0	33.0	8.0
163	18.0	25.0	7.0	20.0	32.0	12.0
172	19.0	trace	trace	24.0	trace	trace
186	19.0	trace	trace	22.0	28.0	6.0
231	21.0	27.0	6.0	24.0	35.0	11.0

diameter of each fungus colony and of the diameter of the yellow, acid zone extending beyond the fungus colony (table 3).

SALTATION TENDENCIES

Variations found in the color, texture, rate of growth, and general physiological behavior of the isolates are evidence of their unstable nature. This conclusion is based upon observation of saltations in some of the isolates. Of the ten cultures studied in detail, No. 90 proved more unstable than the others, or at least the saltants produced by this culture were more obvious. While single-spore cultures were made for all of the isolates as they were acquired, subsequent sub-cultures were maintained by mass transfer of hyphae and spores. It seems probable that less variation would develop in cultures propagated in this manner than in cultures from single spores.

Saltation occurred in culture No. 90 at room temperature when growing on Thaxter's agar. There was a definite wedge-shaped sector of pale gray in the normally dark colony. This pale gray form has been maintained through mass transfer and in only one instance has it appeared unstable in culture. In one sub-culture a sector developed which was darker than the pale form, but not as dark as the parent form. Single-spore cultures from the parent culture were again made and a number of single-spore colonies were observed, all of which were typical of the parent culture.

Reisolations from apple lesions resulting from artificial inoculation, made with a spore suspension from culture No. 90, yielded two cultures that were pale. Single-spore cultures were obtained from these isolates. One remained stable and the other developed a dark sector partially reverting to the parent culture type.

Single-spore cultures were again made from the parent culture. The spores were seeded thinly in the agar and allowed to develop into visible colonies before transferring. An effort was made to select colonies which appeared unlike, and four such colonies were planted in the same petri dish. Two of these colonies were typical of the parent. The third colony was typical in color but of a different texture. The fourth colony appeared abnormal. It had very scant aerial hyphae, the surface being covered by short

upright hyphae, and the general pattern of growth consisted of radiating hyphae with concentric bands.

The instability of culture No. 90 resulted in nine strains which showed certain variations from the parent form. Four of these that differed most are shown in figure 2. Variations in physiological behavior were noted in rate of growth, form, texture, and amount of dark pigment in the fungus colony, and in extent of oxidase production on tannic acid medium. Only one strain varied markedly from the others in the size of the conidia.

These various physiological tests were carried on in an effort to ascertain whether any basis could be found for dividing the isolates into permanent groups, specific or otherwise. Occasionally two or more isolates appeared to be alike or similar in form and behavior. For the most part, however, there were slight variations only which make the group one large series showing gradual variation between extremes that were rather distinct in color, appearance and behavior.

The factors that caused these isolates to have more or less individual differences in appearance, whether due to nuclear fusion somewhere in development or to other unknown causes, seem to have likewise caused the variations in their physiological behavior. This has been demonstrated in the case of the saltants that arose in culture from single-spore isolates. This physiological variation is linked in some way with the ability of the respective strains to assimilate given nutrient media.

It is concluded from the study of the various isolates in general and of the variations found in culture 90 that all may be considered merely as strains of the one species *Phialophora malorum*.

MORPHOLOGY

Considerable variation has been found among the isolates in the texture of the mycelial mat and general appearance in culture. There were likewise differences in the details of hyphal appearance, intensity of color, abundance of conidia and mucous production, size and shape of the phialides and conidiophores, distinctness of the phialide cup and the shape and behavior of the conidia. In some of the isolates the cup was so thin that it was very difficult to discern. In such instances the conidia were no

doubt freed in such manner that there were relatively few torn fragments of membrane at the point of separation.

The isolates included in the detailed study are representative of the large group as a whole. They are basically alike in morphology, especially when observed during the young stages of growth. By comparing the range in size of the conidia (table 4) it is found that a greater variation in size exists within a given isolate than between isolates.

TABLE 4

RANGE IN SIZE OF CONIDIA FOUND IN 10 STRAINS OF PHIALOPHORA MALORUM GROWN ON THAXTER'S AGAR

Serial no.	Source of cultures tested	Range in size of conidia
92	*Sporotrichum malorum* Kidd & Beaumont	4.2–7.8 μ × 1.4–3.6 μ
90	*Sporotrichum carpogenum* Ruehle	3.6–8.4 μ × 1.8–3.6 μ
128	Isolated from fruit lesion—Winesap apple	4.8–7.2 μ × 1.4–3.6 μ
132	Isolated from fruit lesion—Winesap apple	3.6–7.2 μ × 1.2–2.4 μ
158	Isolated from fruit lesion—Winesap apple	3.0–7.2 μ × 1.2–2.4 μ
159	Isolated from fruit lesion—Winesap apple	4.8–9.6 μ × 1.2–2.4 μ
163	Isolated from fruit lesion—Winesap apple	3.6–9.6 μ × 1.2–3.6 μ
172	Isolated from fruit lesion—Winesap apple	3.6–8.4 μ × 1.2–3.0 μ
186	Isolated from soil beneath Winesap tree	4.8–7.2 μ × 1.8–3.0 μ
231	Isolated from cankerous area on Winesap tree	3.6–7.8 μ × 1.2–2.4 μ

Certain other morphological details have been discussed by Gardner (**4**), but details of the process of conidial formation do not appear to have been previously included.

The conidia are extruded from single, terminal, sporogenous cells which Mason (**8**) has defined as phialides (FIG. 3). Typical phialides found in this fungus are short, one-celled, ampulliform, but often unsymmetrical structures. They are swollen through the center, tapering at the base, with a constricted neck and a flared cup at the apex, through which the conidia are formed. The sporogenous cell does not always conform to the above type description, but regardless of shape, the process of sporulation is the same. The first indication of sporulation is the constriction and elongation of the phialide apex. The first conidium formed ruptures the terminal wall of the phialide which apparently has been under tension, and the conidium is cut off and extruded from the phialide. The ruptured end of the phialide is held in the

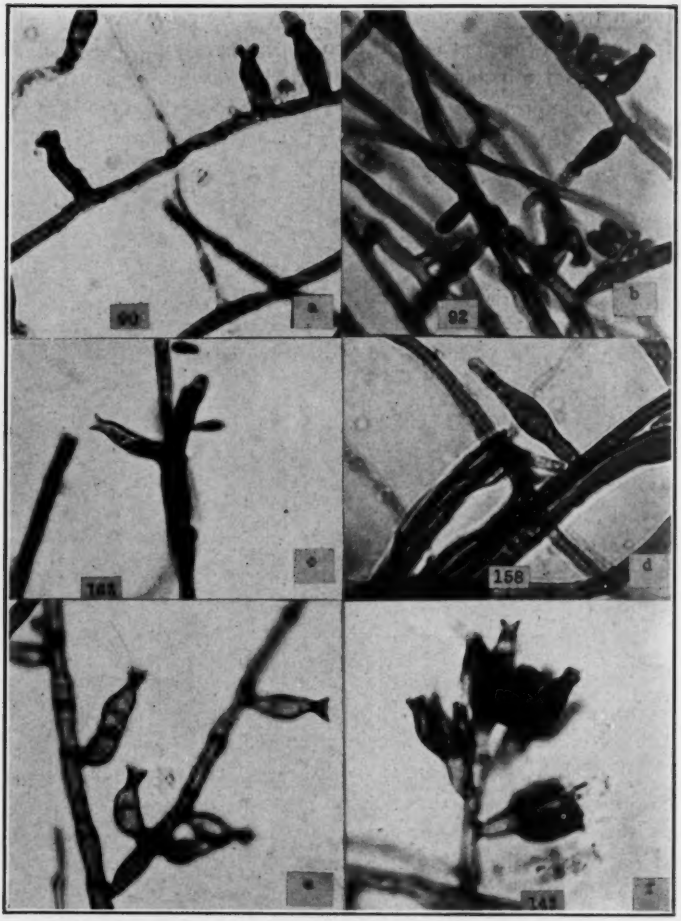

FIG. 3. A comparative study of phialides; magnification 1900 ×; *a*, *b*, *c*, *d* and *f*, strains of *Phialophora malorum; e*, *Phialophora verrucosa*.

shape of a cup by the formation of succeeding conidia, and eventually becomes rigid.

In the normal process, the conidia are never borne within the phialide. The study of stained mounts revealed a pore situated at the base of the cup. It is through this pore that the conidia are

FIG. 4. Phialides of *Phialophora malorum*, greatly enlarged, showing the strands of protoplasm from the sporogenous cells to the developing conidia.

formed. The protoplasm from the mother cell within the phialide, surrounded by a membrane, grows out through the pore and the developing conidium, seated in the open cup grows to maturity (FIG. 4). Thaxter described this process as a proliferation of the sporogenous cell. At maturity the spore wall is formed and the spore separates from the mother-cell connection. It is pushed

out of the cup by the development of the next conidium. It has been impossible to determine accurately all details in the complete process of conidial formation. On some old phialides there appeared to be an accumulation of fragmentary membranes inside the terminal cup as if a portion of the enveloping membrane had been left each time a conidium was extruded. In some of the isolates such fragments were not in evidence. The conidia are extruded continuously in a mucous-like substance until the cell contents of the phialide are exhausted. Conidia produced on the aerial hyphae are held in packets at the tip of the phialides by the mucous. Where sporulation is copious, the masses appear as glistening globules.

TAXONOMY

THE FORM-GENUS SPOROTRICHUM

A brief discussion of the form-genus *Sporotrichum* will make it clear why the apple-rot fungus which has been discussed can not be maintained as a member of it.

Link (**4**) created the form-genus *Sporotrichum* in 1809 and included thirteen species in it. *S. badium*, the only one figured and the first one described, was originally considered as the type for the genus. In 1816, Link (**5**) again treated the genus, adding *Asporotrichum* and *Dematium*. He divided the genus into two sub-genera, *Lysisporium* and *Alytosporium*. In 1818 Link (**6**) revised the genus again, separating *Alytosporium*, which had constituted one of the sub-genera of *Sporotrichum*, thus no longer recognizing sub-genera. *S. badium* had been attached to *Alytosporium* and was no longer regarded as the type species for *Sporotrichum*. His revised key was based upon color, and various colors including black were listed.

Link (**7**) described the genus in 1824 as follows: Hyphae branched, all septate, sporophores without appendages, one celled, not septate and not agglutinated in the thallus (hyphal mat or colony).

Saccardo (**10**) interpreted the genus in 1880 as follows: Light colored, not brown, hyphae branched repeatedly, not erect, hyphae of equal diameter; conidia acrogenous on tips of branches or on small teeth, ovoid or subglobose. He cited *Sporotrichum roseum* and *Sporotrichum virescens* as examples.

Engler and Prantl (2) describe the genus as follows: Hyphae richly branched, widely spreading and all appressed, conidia apical, at tips of branches or on short sterigmata, mostly single, egg-shaped or spherical. Saprophytes, more than 120 species, of which almost half are assigned to Central Europe. A large number of these are doubtful. They figured *S. roseum* and *S. geochroum*.

There are probably three outstanding reasons for the confusion surrounding this form-genus: (1) The original inclusion of dark or black-colored fungi; (2) lack of an adequate description, especially of the manner in which the conidia are formed; (3) the uncertainty over what is to be considered the proper type species and the shifting from one species to another in an attempt to establish a type.

This review of the genus *Sporotrichum* is sufficient to show that the apple-rotting fungus, which has been designated as *Sporotrichum malorum* Kidd & Beaumont, can be excluded from the genus upon such characteristics as dark color in the hyphae, poorly to moderately branched hyphae, and in the mode of conidial formation.

It is therefore evident from the preceding discussion of the physiological behavior and morphology of the several strains, including Ruehle's "*Sporotrichum carpogenum*," that but one variable species is concerned and that it may properly be designated and given an amended description as follows:

PHIALOPHORA MALORUM (Kidd & Beaum.) McColloch, Phytopath. **32**: 1094. 1942.

Sporotrichum malorum Kidd & Beaum., Trans. Brit. Myc. Soc. **10**: 111. 1924.

Sporotrichum carpogenum Ruehle, Phytopath. **21**: 1144–45. 1931.

Hyphae first hyaline, then fuscous to brown, septate, 1–4 μ in diameter, which show a tendency to adhere in rope-like strands, moderately branched, resting hyphae sometimes becoming moniliform; terminal and intercalary chlamydospores; conidiophores composed of specialized sporogenous cells, typically vase-shaped, 5–10.8 μ long, arising laterally, terminally or in clusters from the hyphae; conidia hyaline to fuscous, sub-globose to elliptical,

unicellular, 4.2–7.8 × 1.4–3.6 μ, formed through a cup-like apex by a proliferation of the sporogenous cells, separated from the cells at maturity and held in a mucous mass.

Distribution: In surface soil and on cankerous apple wood in Virginia. Causing decay of apples in the United States and Great Britain.

ACKNOWLEDGMENTS

The author expresses thanks to Dr. Charles Thom for assistance in the identification of the fungus; to Miss Edith Cash and Dr. D. H. Rose for translations in connection with the literature reviewed; and to Mr. John A. Stevenson for many helpful suggestions during the preparation of the manuscript.

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A NEW GENUS OF SMUTS

M. J. THIRUMALACHAR

(WITH 11 FIGURES)

Heptapleurum venulosum Seem. is a glabrous tree common in the mixed forests of Mysore. It bears in season small orange colored fruits which give it a very characteristic and attractive appearance and the tree is often grown therefore in ornamental gardens. One such tree growing in Lalbagh at Bangalore was found to be heavily infected by a smut. The smut is principally fructicolous (FIG. 2) but it also attacks the stems, the petioles and the leaves. In the attacked fruit, the embryo and the endosperm get replaced by a pulverulent mass of spores which are held together by the dried pericarp. Partially infected fruits are also common.

On the stems the smut is usually confined to the tips where the apical meristem gets displaced by the sorus which at first is subepidermal (FIG. 1). On the petiole, especially at the point of insertion of the leaflets, the smut forms black, wart-like (FIG. 3) sori. The sori on the leaves resemble the telia of *Nyssopsora Thwaitesii* (Berk. & Br.) Sydow, which is also common on this host.

For the study of the development of the sori and the spores, the best material proved to be the leaves and the pericarp of the fruits. These were fixed in Allen's modification of Bouin's fluid and also in Navashin's fluid as modified by Karpechenko. Embedded material was cut 6 to 10 μ thick and stained with Haidenhan's iron-alum haematoxylin or Newton's gentian violet.

The sori first appear in the intercellular spaces and gradually widen by the distension of the host cells. In a mature sorus (FIG. 10) two types of spores have been noted: (1) small two-celled oblong-ovate spores with rich cell contents and deep chocolate brown color, (2) large one-celled spores which are hyaline or pale yellow with degenerated cell contents and hence presenting a vacuolate appearance. The spores of the first category remain,

in many cases, one-celled due to lack of formation of the septa but they can easily be distinguished from the second category of spores described above. These latter are very much larger in size and their hyaline or pale yellow color distinguishes them from the mesospore-like spores that are also one-celled. The sori are thus heterosporous, a feature not so far recorded in the Ustilaginales.

Prior to the formation of spores the mycelium in the intercellular spaces becomes closely septate (FIG. 6) and the hyphal cells which are binucleate get gradually rounded (FIGS. 4 and 5). The sorus at this stage resembles the sorus of *Entyloma* but as development proceeds some of the cells become slightly ovate and richer in cell contents. A thick epispore then develops and a septum is formed (FIG. 7). The mature spores are deep chocolate brown with a minutely but deeply pitted wall which gives their edges a serrate appearance. Due to the lack of formation of septa these spores sometimes become one-celled, though in other respects they are identical with the two-celled spores (FIG. 8). Other cells in the meanwhile greatly enlarge in size, do not form a septum or develop any color. The cell contents of some of them degenerate, finally becoming vacuolate (FIG. 9).

The spores were germinated only with difficulty in plain agar in petri dishes. Germination was very capricious and took place after a lapse of much time. Germ tubes were put forth by the two-celled spores as well as by the one-celled pale yellow spores and the deeply chocolate brown spores (FIG. 11), but the hyaline vacuolate spores failed to germinate. Probably they are to be considered as sterile cells. Germination is by the formation of a septate promycelium on which sporidia are formed rather infrequently, both laterally and terminally. This mode of germination conforms to the type characteristic of the Ustilaginaceae. A peculiar feature noted in the germination of the spores of this smut, which has not been reported in any other genus of the order Ustilaginales, is the formation of a constrictor cell between the cells of the promycelium. This cell apparently helps to sever the promycelial cells easily and the separated promycelial cells probably assume the role of sporidia. The sporidia are globose, thin-walled and hyaline.

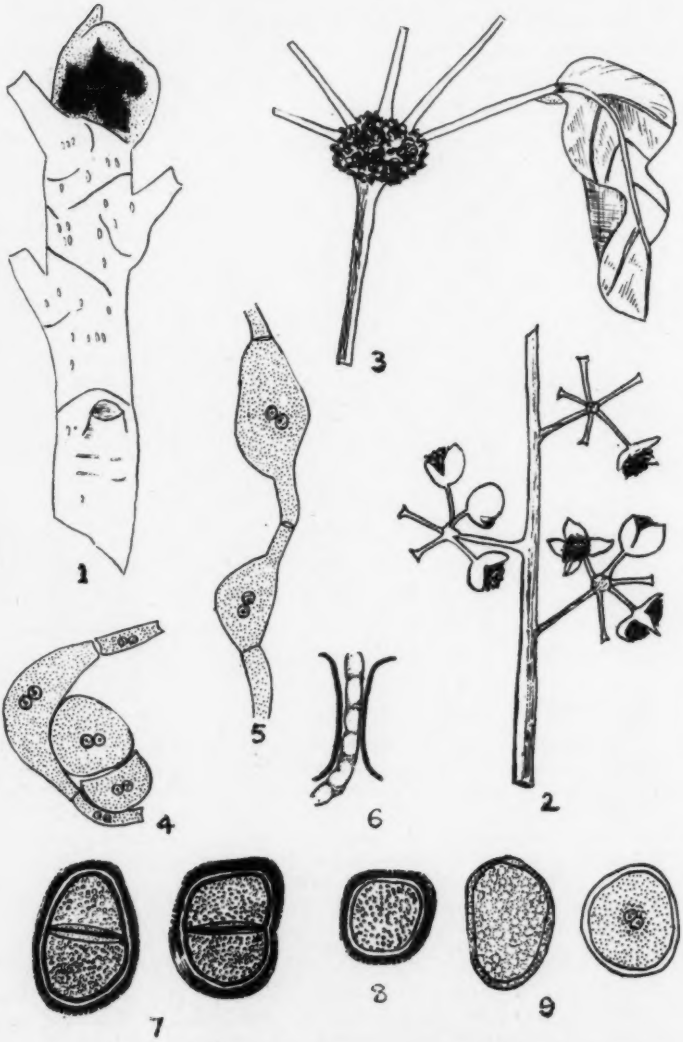


FIG. 1. Infection of twig, ¾ nat. size; 2, infected fruits, nat. size; 3, infected petioles, ¾ nat. size; 4 and 5, developmental stages of spores, × 1800; 6, septate hypha, × 1800; 7, mature 2-celled spores, × 1800; 8, 1-celled mesospore-like spore, × 1800; 9, hyaline 1-celled spores, × 1800.

Two-celled spores, as distinct from spore-balls, are present in *Schizonella*, *Mycosyrinx* and *Schroeteria*. Species belonging to the genus *Schizonella* attack species of the genera *Carex* and *Elymus* and form their sori on the leaves. The spores which are two-celled are roughly dumbel-shaped but the two cells are easily detachable. In *Mycosyrinx* which attacks species of *Cissus* and *Vitis* and which forms its sori in the peduncles, the sori are enclosed in a double peridium and the two cells of the spores are attached by an isthmus-like structure but they easily get detached. In the genus *Schroeteria* which has been doubtfully placed in this Order, both two- and three-celled spores occur but on germination they form a long septate germ tube at the apex of which chains of conidia are formed. Neither Dietel (**1928**) nor Clements and Shear (**1931**) place it among the smuts though doubtfully placed in the Tilletiaceae by Lindau (**1914**) and Liro (**1938**).

The unusual features which the smut on *Heptapleurum venulosum* possesses, and which are not found in any other genus of the Ustilaginales, entitle it to a place in a new genus to which the following name, for Dr. B. B. Mundkur, is given. These features are heterosporous sori, constrictor cells between the promycelial cells, viable one- and two-celled spores.

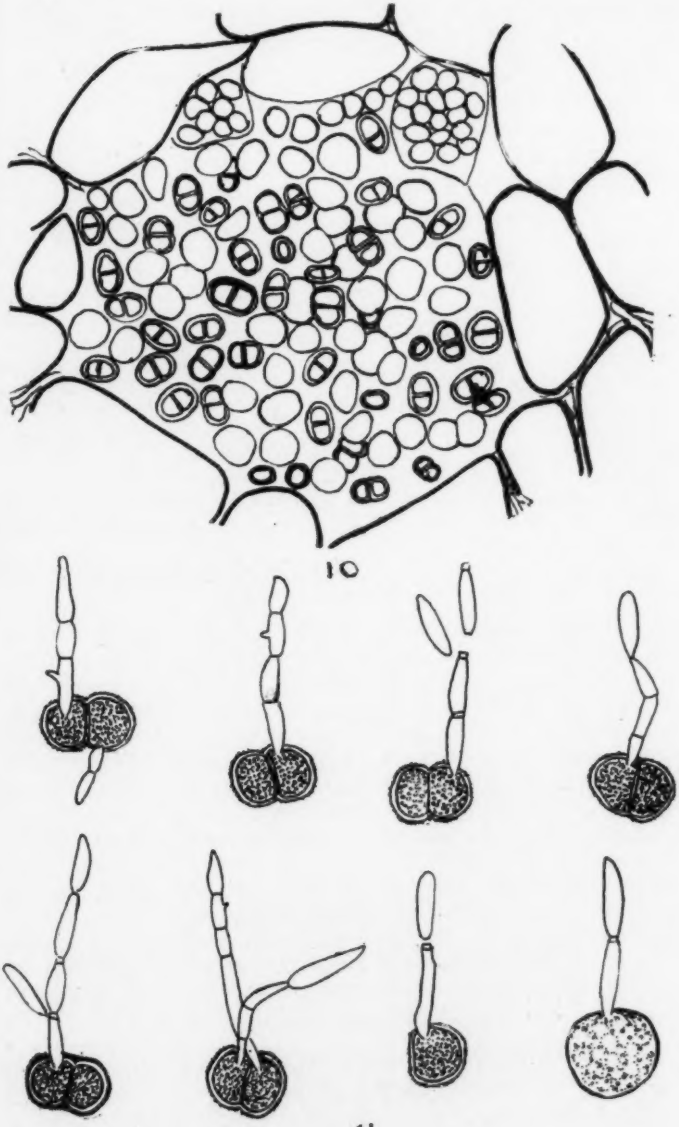
Mundkurella gen. nov.

Sori in fructibus, caulibus, petiolis, atque foliis, heterospori, duplici sporarum genere praediti: (1) sporis unicellularibus, quarum quaedam copiosa inclusione atque lateritio-brunnea colore ditatae, quaedam vero pallide flavidae vel hyalinae sunt; (2) sporis bicellularibus, copiosa inclusione atque lateritio-brunneo colore praeditis. Germinatio per septatum promycelium; cellulae constringentes inter promycelii cellulas inveniuntur; plurimae sporae unicellulares vel pallide flavae, ut plurimum steriles.

Species typica: *Mundkurella Heptapleuri* Thirumalachar, *Heptapleuri venulosi* parasitica.

Sori in fruits, stems, petioles and leaves, heterosporous with two kinds of spores: (1) one-celled spores some of which with rich cell contents and chocolate brown colour and others pale yellow or hyaline; (2) two-celled spores with rich cell contents and chocolate brown colour. Germination by formation of septate

FIG. 10. Sorus showing heterosporous condition, × 400; 11, spore germination, × 800.



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promycelium; constrictor cells between cells of the promycelium present; large number of one-celled hyaline or pale yellow spores, as a rule, sterile.

Type species: *Mundkurella Heptapleuri* Thirumalachar on *Heptapleurum venulosum* Seem.

Mundkurella Heptapleuri sp. nov.

Sori heterospori, in fructibus, caulibus, petiolis et foliis; in fructibus endospoermum atque embryo omino, raro partim tantum, destruuntur, sola testa intacta atque sporis pericarpio inclusis remanentibus. In caulibus sori subepidermales, omino evertentes meristematicas cellulas ex apice, earumque locum occupantes; in petiolis atque foliis nigras verrucas efformantes. Sporae duplicis generis; unicellulares subglobosae vel ovatae penitus lateritio-brunneae, flavidae vel hyalinae, sporae lateritio-brunneae 8.5–13.5 μ diam., sporae hyalinae magnitudinis 12–29 μ diam.; sporae bicellulares penitus lateritio-brunneae, inclusionibus copiosis atque granularibus, ovato-oblongae, 13.7–22 $\times$ 10.3–13.5 μ; episporium crassum, profunde atque minute excavatum, margine apparenter serrato. Germinatio per septatum promycelium, quod sporidia lateralia atque terminalia efformat; promycelium apparentiae insolitae, et ornatum cellulis constringentibus inter cellulas promyceliales; singulae bicellulares sporae et circiter 30 ex unicellularibus sporis viabilis; ceterae manifeste steriles.

Typus lectus a. cl. M. J. Thirumalachar super Heptapleurum venulosum Seem. 15–8–1942, Lal-Bagh, Bangalore, Indiae Or.

Sori heterosporous, in fruits, petioles, stems and leaves; in fruits endosperm and embryo completely and rarely partially destroyed, with only testa remaining intact and spores held together by the pericarp; in stems sori subepidermal, completely replacing the meristematic tissues at the apex; in petioles and leaves sori forming black warts. Spores of two kinds: one-celled spores subglobose or oval, chocolate brown, pale yellow or hyaline, chocolate brown spores measuring 8.5 to 13.5 μ and hyaline spores 12–29 μ in diameter; two-celled spores deep chocolate brown with rich granular contents, ovate-oblong, 13.7–22 $\times$ 10.3–13.5 μ; epispore thick, deeply and minutely pitted giving the edge a serrate appearance. Germination by the formation of a septate promycelium forming terminal and lateral sporidia; promycelium of unusual shape and provided with constrictor cells between the promycelial cells; all two-celled spores and about 30 per cent of one-celled spores viable, the rest manifestly sterile.

Hab. on *Heptapleurum venulosum* Seem. at Lal-Bagh, Bangalore, Coll. M. J. Thirumalachar, 15–8–1942 (Type); Coll. B. G. L. Swamy, 10–8–1942. Type deposited in the *Herb. Crypt. Ind. Orient.* New Delhi.

The writer wishes to express his gratitude to Dr. B. B. Mundkur, Imperial Agricultural Research Institute, New Delhi, for examining the smut material and for confirming the conclusions arrived at above, and to Dr. L. N. Rao, Professor of Botany, University of Mysore, for the help and encouragement given by him. Rev. Father H. Santapau, S.J., Ph.D., Professor of Botany, St. Xavier's College, Bombay, kindly rendered the diagnosis of the new genus and the species in to latin.

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CONTRIBUTION TO THE EPIDEMIOLOGY OF TINEA CAPITIS. III. SOME DIAGNOSTIC PROBLEMS IN TINEA CAPITIS

TIBOR BENEDEK[1]

In two previous publications (1) a study of tinea capitis in a dispensary [2] was presented and (2) the epidemiology of this fungous infection as a public health problem in family and school [3] was investigated.

How timely these investigations were, suffice to point at the severe outbreak of tinea capitis in the Eastern States at the present time. The daily press carries alarming reports of the spread of tinea capitis on an epidemic scale among school children in Pittsburgh, Pa., Philadelphia, Pa., and New York, N. Y., as far as it came to my knowledge.

The imminent danger of the spread of tinea capitis on an epidemic scale as it was experienced during and after the first World War in Central and Eastern Europe presented itself in this country much earlier than it could be expected. I underscored the possibility of an epidemic, even pandemic outbreak of tineal infection since the current conditions in this country present all the requisites for such an occurrence.

Despite the great efforts for the improvement of diagnostic procedures there are still some problems which need clarification. In view of the present emergency I propose to present the standard procedure of investigations applied in every case of tinea capitis in our laboratory. By passing as the necessity may arise controversial problems will be discussed. Standard procedure of investigations followed the subsequent scheme:

[1] From the Laboratory of Mycological Research, Department of Dermatology, Chairman: Dr. Edward A. Oliver, Northwestern University Medical School, Chicago, Ill.

[2] Benedek, T. & Felsher, I. M. Epidemiology of tinea capitis. I. A study of tinea capitis in a dispensary. Arch. Dermat. & Syph. **49**: 120–123. 1944.

[3] Benedek, T. Epidemiology of tinea capitis. II. Tinea capitis as a public health problem in family and school. Urol. & Cutan. Rev. **47** (7): 416–432. 1943.

1. Clinical examination.
2. Examination of the scalp and face in Woodlight (fluorescence).
3. Demonstration of fungi on direct microscopic examination.
4. Culture of fungi from hairs and scales.
5. Identification of the growth with determination of the species.

1. THE CLINICAL EXAMINATION: THE CARRIER PROBLEM

The first step to establish definitely the clinical diagnosis of *any* tinea capitis leads us to a problem of paramount importance, unknown in the past, very little discussed in the whole literature.

This problem is the *existence of carriers*. A "carrier" is a person who, without symptoms of a communicable disease, harbors and disseminates the specific micro-organisms. Carriers of fungal organisms in tineas cannot be detected by the most thorough clinical inspection. They do not reveal any clinical signs or symptoms, they simply present normal healthy scalps.

In order to detect carriers we always need some special tool to expose them. In typhoid carriers, for instance, this tool is the bacteriological method. Without proper bacteriological examination their existence cannot be detected and the carrier is a permanent danger of infection for the immediate surroundings and even for the larger community.

We face, mutatis mutandis, a similar problem dealing with tinea capitis. Between typhoid carriers and tineal carriers, however, there is a great difference. The primary infection in typhoid never goes unnoticed. It is promptly revealed by rather stormy clinical signs and symptoms. In tinea capitis, on the other hand, the primary infection is always insidious without signs and symptoms. Therefore, such a carrier is a potential source of infection from the moment as the fungus took foothold in the first hair follicle. Such a carrier potentially might spread the infection for weeks and months before the first sign of an infection can be detected clinically on his own scalp.

Due to these differences, the only tool which is absolutely reliable in the earliest detection and elimination of these tineal

carriers is not the microscope or the cultural method, *but the fluorescence phenomenon of the Woodlight.*

The importance of the Woodlight in the earliest detection of carriers, in the control of progress of our therapeutical measures, and in the determination of a final cure in tinea capitis must eventually be understood by all those dealing with this condition. Unfortunately, some of the largest American texts on Dermatology do not even mention the Woodlight and its paramount significance in the fight against tineas of the scalp. Some deal with this problem in an entirely insufficient manner. This is all the more surprising because R. W. Wood, an American physicist of world renown, gave this important tool to dermatology as one of the foremost single diagnostic tools almost a quarter of a century ago. The significance of the Woodlight as a diagnostic tool in tinea capitis is only comparable with that of darkfield microscopy in syphilis. Benedek gave recently a graphic demonstration of the importance of Woodlight in a publication on luminography (**1**).

To my knowledge, Davidson, Gregory and Birt (1934) (**2**) were the first who clearly recognized the carrier problem in tinea capitis. They examined 170 patients and contacts for ringworm of the scalp. On clinical examination, 31 out of 170 (18.2 per cent) were positive. All gave the positive test in Woodlight. However, among the 139 clinically negative cases seven (5 per cent) revealed positive fluorescence. They promptly concluded that the findings of fluorescent hairs on seven patients who were clinically passed as negative is the outstanding feature of this examination. They added furthermore that observation of kitten-carriers (**3**) throws new light on the epidemiology of ringworm of the scalp and *postulated* the possibility of human carriers of the disease.[4]

2. EXAMINATION OF THE SCALP AND FACE IN WOODLIGHT

The "clinical examination" played only a secondary role in the procedure to establish or to exclude the diagnosis of tinea of the scalp. Clinical inspection was, however, always the first step to

[4] Surprisingly even the latest publications (Lewis and Hopper (**8**), Livingood and Pillsbury (**16**)) do not deal with this problem.

start with, even though for nothing more than to be even more impressed by the revelation of the extent of fungus infection of the scalp and to bring home to our students in the class room and to the parents of our little patients, the efficiency and absolute necessity of the inspection of the scalp in Woodlight. The fluorescence usually revealed many scattered little tufts of hairs or even many isolated single hairs all over the scalp beside and beyond the clinically recognizable one or more smaller or larger foci.

I always insisted on the presence of the parents at the inspection of the scalps of our little patients in Woodlight. This served primarily not the purpose to keep them quiet or without fear in a strange and completely dark room. Our intention was to impress the parents, to win their collaboration and to induce them in that way to bring the whole family (not infrequently, we have had families with 5 to 9 children) for examination. We did not fail in any case.

From the very start of these investigations efforts were not restricted to the exclusive management of the individual case as dispensaries usually do, or at least did in the past. The survey was undertaken from the beginning from a broad, epidemiological point of view. The single case was only the lead, the clue to be followed in order to trace down the individual case to the source of infection; to establish the carriers in the family and to carry on the search for possible farther spread of the infecting agent into large communities like schools, kindergartens, asylums, etc.

Both in the dispensary cases and in a special investigation carried out in one whole public school with nearly 3,500 pupils (**4**, **5**) the large percentage of carriers in the families and in the school room, was a great surprise. The number of carriers was 25 per cent in the dispensary material and 53 per cent in the school examined. These figures reveal the absolute necessity and paramount efficacy of the inspection of the scalp in Woodlight.

Just recently Cleveland (**32**) made a similar statement that as time passed he has been struck with increasing force by the observation that a large proportion of cases which have presented no clinical signs whatever, have been found nevertheless to be "Woodlight positive."

Concerning the problem of carriers I may give here one most illustrative case. A well developed negro girl, aged 17, came one day to the dispensary on account of a pityriasis versicolor.[5] After demonstration of the extent of this saprophytia on the body in Woodlight, I proceeded to show how normal black hairs fluoresce is a dusty grayish color. As the Woodlight fell on the luxuriant black hairs of the patient, beside the dusty-grayish fluorescence of the normal hair and beside the bluish-purplish color of the grease she had in some part of her hairs, she revealed ***one single hair*** in the central part of the hair glowing in a beautiful emerald-green color. It was unmistakably an infected hair. This single hair was epilated by hand under Woodlight. It was invaded by the fungus up to two cm. above the hair follicle. A portion of this hair served for microscopical examination, revealing the presence of microsporum. The larger portion was used for culture. It yielded a pure culture of *M. Audouini*. She had infected siblings at home and she became a carrier despite her age and overripe development.

Microsporum hair fluoresces in Woodlight with a beautiful, saturated copper-green or emerald-green color. Anthropophilic and zoöphilic species give the same fluorescence in infected hairs. They thus cannot be told apart by the physical means, just as they cannot be separated in foretelling the species by microscopical examination. In this respect only the culture decides.

Since there are still some controversial views about the value and efficiency of Woodlight as a diagnostic tool in tinea capitis in the literature, a few critical remarks have to be added.

First of all, no difficulty was experienced to detect carriers in Woodlight even though the hair (like in many negro girls and boys) was richly greased. The white-bluish-purplish color of the

[5] We usually demonstrate this saprophytia to our students under Woodlight, because we want to call their attention to two facts, that (1) many patches of pityriasis versicolor can be seen scattered all over the body, often on neck, face, forehead, in areas which cannot be seen or detected at the closest inspection at the best daylight; and (2) to give the simple convincing reason of the up to date highly mysterious recurrences of this in itself harmless infection. In pityriasis versicolor like in tinea capitis we treated in the past only the clinically conspicuous, visible patches and let enormous numbers of parasites untouched, therefore the prompt recurrence.

plain grease contrasted even more with the emerald-green color of the microsporum infected hair.

Berde (6) called attention to the fact that sometimes cases of tinea microsporina and favosa, though clinically and mycologically proved beyond doubt as such, did not reveal either the usually intensive or characteristic fluorescence. He made this observation in three sisters infected with microsporum. Two of them revealed the usual sparkling fluorescence, the third was, however, negative. He attempted to find the cause of this peculiarity not in the species of the infecting agent, but in some "constitutional deviation" of the scalp from the normal or in the influence of some contaminant or in applied therapeutical means. Furthermore, this author assumed that desquamation of different (not fungal) origin, diverse chemical agents brought to the hairs incidentally or intentionally may elicit the same fluorescence as microsporum, favus, etc. He disagreed with Cleveland (7) who considered Woodlight as a tool of paramount importance in the detection of ringworm of the scalp.

I feel I have the right to take the stand in this controversial matter. I feel I can insist upon the correctness of these observations and the conclusions based upon and derived from them. I have observed a series of over 4,000 individual scalps; a large enough sequence, if not the largest single series ever observed, to have the proper safety margin of the laws of big numbers.

I definitely disagree with the statement of Berde that desquamation of different (not fungal) origin on the scalp may elicit the same or similar fluorescence as microsporum, favus or endothrix trichophyton. The only desquamative process that may raise some doubt or confusion *at clinical inspection* is the excessive form of *pityriasis capitis* or a mild *psoriasis*. We had several children under observation with these conditions in which clinically one could not exclude the possibility of a tinea capitis. In every case, one inspection under Woodlight positively ruled out the presence of any tinea. Microscopical and cultural controls always substantiated the correctness of the inspection, and the absence of any fungi. Seborrheic or psoriatiform scaling of the scalp reveals a chalky-white fluorescence with hairs not fluorescing at all. There is no possibility for confusion of the emerald-green color of

a microsporum hair with the chalky-white color of seborrheic scaling.

Among the *chemicals* plain grease or oil (not medicated) having an explicit bluish-purplish color in Woodlight does not interfere with any fungal fluorescence.

Some chemical agents, however, may extinguish the fluorescence entirely. Salves containing salicylic acid may cover up the emerald-green color of microsporum completely by their own bluish-white fluorescence. This fact, however, cannot detract anything from the usefulness of the Woodlight as a diagnostic tool. If the salve was applied before, it was first removed by soap and water. Afterwards one never failed to see how far the infection is under control.

Rubbing off with benzine or alcohol the conspicuously infected areas of the scalp one could ascertain that these chemical agents do not influence the fluorescence in any way. Tincture of iodine, however, extinguishes the most intensive fluorescence momentarily.

Eventually I have to discuss a remark of Berde that sometimes cases of tinea microsporina and favosa did not reveal either the usually intensive or characteristic fluorescence. He assumed a "constitutional deviation" of such an infected scalp. Berde missed here a very important observation.

As to the plain fact it is true that sometimes in exactly the most severe cases of tinea microsporina the fluorescence is unusually dim. An inexperienced observer could definitely judge it as negative. Such extensive cases covering practically the whole scalp were seen by me in a few instances where I made a similar observation as Berde. These cases, however, do not constitute any puzzle or any kind of difficulty for clinical diagnosis. I was certainly puzzled observing these extensive infected areas in which here and there one or two single hairs gave the perfect emerald-green fluorescence, but otherwise the whole infected scalp was dimmed, almost blacked out.

I found the definite physical reason of this dimming or blacking out of fluorescence in tinea microsporina. I emphasize that I came across this peucliarity only in extensive cases which, of course, lasted one to two years, in one case over four years. I

found the solution of this interesting problem at the microscopical examination of a very great number of hairs and hairstumps from different parts of these scalps. It was found that hairs and hairstumps from dimmed or blacked out areas *do not possess any spore sheaths around the hair shaft.* In comparison the few scattered single hairs fluorescing in perfect emerald-green color revealed the existence of an intact spore sheath. Cultures from epilated hairs under Woodlight gave from both kinds of hairs exactly the same culture of microsporum.

The lack of the spore sheaths causes the dim fluorescence or its blackout and not a "constitutional difference" in the scalp of the infected individuals.

In cases of tinea trichophytina (endothrix) and tinea favosa Woodlight served a similar useful purpose in detection of infected single hairs or tufts of hairs outside the clinically anyway conspicuous areas. Endothrix trichophyton fluoresces on the scalp as a fine pale blue hue, favus hairs reveals a beautiful canary-yellow color. If one would have needed to be convinced of the usefulness of the inspection of the scalp in Woodlight two favus cases in the series would have sufficed.

I saw one boy, age 13, and a girl, age 11, in whom favus of the scalp persisted for 11 and 8 years respectively.[6] They have had ever since a "proper" medical care and their favus could not be eradicated. The Woodlight solved this problem. Beside the clinically visible foci there were numerous infected hairs, single or in small tufts, scattered over the scalp, far away from the clinically visible lesions. These hairs could be seen as infected ones only under Woodlight and not otherwise. That is the reason for the endless duration of a favus infection of the scalp because as in these cases only the visible areas were treated and not all the infected hairs.[7] I gave a similar reason for the continuous recurrence of pityriasis versicolor on the glabrous skin.

I observed three cases with large boggy kerions caused by *Achorion gypseum*. Beside the kerion, a few scattered hairs gave a dim pale bluish fluorescence.

[6] I thankfully acknowledge the referral of these two cases for mycological examination by our staff member, Dr. S. J. Zakon.

[7] Even after total epilation only the inspection of the scalp under Woodlight can reveal whether the therapeutical effort was successful or failed.

3. DEMONSTRATION OF FUNGI ON DIRECT MICROSCOPIC EXAMINATION

After clinical inspection and careful observation of the scalp under Woodlight the diagnosis was always verified by direct microscopical examination of the hairs. An absolute parellelism was found in microsporum, favus, endothrix trichophyton hairs between the fluorescence in Woodlight and the presence of fungal elements under the microscope. On the other hand, microscopically negative hairs do not fluoresce at all. Therefore the final decision for the discharge of the patients was made not by means of microscopical examination but by the fluorescence test in Woodlight.

I absolutely discarded the use of potassium hydroxide (KOH) for the microscopical examination of hairs and scales. Instead I use one of the following solutions:

(1) Chlorallactophenol:

Rx chloral hydrate cryst.	20.0
acid. carbol. cryst.	10.0
acid. lactic. pp.	10.0

S. mix and dissolve on waterbath.

(2) Salicylated chlorallactophenol:

Rx chloral hydrate cryst.	40.0
acid. carbol. pp.	40.0
acid. lactic. pp.	20.0
sod. salicylate	10.0

S. mix and dissolve on waterbath.

If staining of the specimen is desirable (usually not for the routine examination) *the liquid of amman* with cotton blue (C_4B Poirier) can be recommended:

Rx acid. carbol. cryst. pp.	10.0
acid. lactic. pp.	10.0
glycerin	20.0
acqua dest.	10.0
cotton blue	0.05

S. mix and dissolve the first four ingredients in the order given on waterbath, then add cotton blue.

I cannot agree with Lewis and Hopper (**8**) who gave also their special attention to this matter that "For ordinary routine use we

have not found anything better than a 10 per cent solution of potassium or sodium hydroxide." [8]

Using even a 10 per cent KOH or NaOH (let alone a solution of 40 per cent!) the solution quickly absorbs CO_2 from the air at the opening of the container whatever it may be. The result is the formation of potassium or sodium carbonate (K_2CO_3 or Na_2CO_3) as a crystalline sediment, reducing at the same time the effective hydroxide almost to nil. Such a solution, I need not emphasize, is absolutely useless. This is the chemical side of the problem.

The mycological side, however, is even more important. Using KOH for clearing of the material (hairs or scale) even the natural fat of the hair, let alone the grease often applied to them, is most disturbing. If the cold process is used for clearing, the crystallization of the hydroxide or (mostly) the presence of carbonate crystals mingled with fat is highly disturbing. If the hydroxide preparation is even gently heated the saponification of the grease can make the observation, particularly for less experienced examiners, very difficult. Last but not least, if such a preparation is heated a little bit more energetically the whole structure of the hair—is entirely destroyed, the hair appearing like "exploded."

Removing the fat previously by acetone, alcohol or ether is tedious, time consuming and using a chlorallactophenol formula, entirely superfluous job. Furthermore hydroxide preparation cannot be stained and only with difficulty conserved. All these difficulties are met with and promptly overcome using one of the above formulas.

The advantages (I never found any disadvantages) of chlorallactophenol formulas are the following: (1) it is a cold process, no warming or heating is necessary; therefore the structure of fungal elements and their natural relation to the host cells are perfectly preserved; (2) fat adherent to hairs or scales does not disturb the picture because there is no saponification; (3) animal tissue (hair, scale, pus cells) becomes absolutely transparent leaving the vegetable elements (spores, mycelium, etc.) in full conspicuity; (4) there is no influence of CO_2 from the air; (5) the preparation

[8] Current dermatological texts give usually the advice to use a 40 per cent KOH solution. Such advice can be traced to some ancient outmoded source of information and uncritical compilation.

as it is made without any further time consuming manipulation can be conserved by simply framing it by wax or resin. Formula 1 can be used for scales and lesser pigmented hairs; formula 2 is the best for heavily pigmented (negro) hairs; formula 3 can be used at any time if staining of a specimen is primarily desirable.

I referred to the repeated observation in long-standing cases of microsporum infection of the scalp that hairs do not show any spore sheath, nonetheless, the hair shaft is filled with mycelium. In these not so rare instances the question arises whether the observer is dealing with an endothrix (*Trichophyton*) infection or not. There is a sure objective sign to differentiate the (in reality) microsporum hair from a supposedly endothrix hair. This sign is given in the state of the hair cuticle. In a typical endothrix infection, the cuticula of the hair shaft is absolutely smooth, intact. In the case of a microsporum hair without spore sheath the cuticula is roughened up, torn up here and there and its continuity is in many places disturbed, of course, without seeing any spores outside and along the hair shaft.

I made another nteresting observation in long-standing cases of microsporum infection. I have had several boys under observation (white boys, with blond hairs) who showed one from 3 to 5 cm. large round patch of microsporum infection on the occiput. Due to circumstances beyond our control these boys disappeared after the first few consultations for many months from observation. When eventually seen again 10–12 months after the first consultation I surprisingly made the observation that (1) the infected patch remained isolated; no spreading took place; (2) the hairs were invaded 2–3 cm. long with mycelium showing up with whitish-bluish fluorescence, and not revealing microscopically any spore sheath. The culture, however, carried out with great care, revealed that these hairs were invaded by *M. Audouini.*

It seems to me that in long standing infections there is some balance (immunity of the scalp?) between fungus and the host developing and very little or no tendency of spreading; further that the fungus (*M. Audouini*) changes somewhat its relationship to the hair, remaining entirely "endothrix." These hairs are usually long as healthy hairs (I made the similar observation in

both cases of favus), they do not break off, they do not reveal cross breakage of the shaft under the microscope along their whole length and they can be discovered as infected, exclusively under Woodlight.

These cases are the worst carriers. Clinically they show not the least change or deviation from normal hairs, they spread the infection nevertheless, judged by the intensive growth of the fungus from these peculiar hairs in the culture.

4. CULTURE OF FUNGI FROM HAIRS AND SCALES FROM STANDPOINT OF EPIDEMIOLOGY

As is well known, the differentiation of so-called "species" of dermatophytons, *Microsporum*, *Trichophyton*, *Epidermophyton* was fundamentally based in Sabouraud's system, beside their relationship to the hair and epidermis and certain general microscopical features,—on their aspect and appearance in the giant culture. The basis of comparison was given by Sabouraud's famous "milieu d'épreuve," made up with raw French glucose and maltose and pepton Chassaing. It was always understood that these carbohydrates were chemically impure carrying with them qualitatively and quantitatively an entirely unknown amount of "accessory" material which possibly being vitamins in character, gave rise to luxuriant giant cultures. The peptone used was itself of an unknown and unsteady compound.

The luxuriant growth and the surface configuration (the most important differentiating characteristics for the giant culture in Sabouraud's system beside their color) could not be well reproduced in an identical way by any other combination of peptones and carbohydrates of German, French, Hungarian or American origin.

Medical mycologists all over the world willingly accepted Sabouraud's "milieu d'épreuve" as the international comparative nutrient medium, while botanical mycologists like Linder (**9**) [9]

[9] Linder (l.c.p. 455–456) said: "It is my firm conviction that the almost universal use of Sabouraud's agar has done more to confuse taxonomy than any single factor. Peptone or peptones and sugar in agar, as used in the past, are not only unbalanced and do not furnish all the elements necessary for the normal growth of the fungi, but when these substances are supplied, they are in too great concentration. Test-tubes do not furnish a normal habitat, but when to this is added unfavorable nutritional factors, there is little wonder that the organisms go wild and produce all sorts of mutation."

frowned upon it for many good reasons. It is, however, well known to those who had opportunity as the writer, to work with Sabouraud's "milieu d'épreuve" for many years and had the opportunity to compare in the long run giant cultures of the same species on different batches of the French carbohydrates and peptones, that even this milieu d'épreuve proved to be inconstant to a lesser or a greater degree in reproduction of the size, shape, configuration, color of the same species in the giant culture.

Up to the outbreak of the first World War French material was easily available all over the world. The war and subsequent difficulties in the international exchange of goods made in this respect a definite break, which chasm is deepened to an indefinite interruption. Sabouraud's "milieu d'épreuve" is past history.[11]

Yet from the standpoint of epidemiological research a comparative nutrient medium was obviously of paramount importance. Weidman and McMillan (1921) in the United States, G. Pollacci (1922) in Italy, O. Grütz (1923) in Germany, Goldschmidt (1924) in England tried to evolve a comparative nutrient medium from national resources to replace Sabouraud's original milieu d'épreuve but without stretch of imagination the giant cultures of the same dermatophyte could be hardly compared with the pictures published in Sabouraud's magnificent volume "Les Teignes," in 1910.[11]

Based on his milieu d'épreuve, Sabouraud established beside the type species in *Microsporum*, *Trichophyton*, a great number of "satellites" as valid species based mainly on slight differences of the giant culture in color, configuration of the surface, rate of growth, etc. These satellites are, however, no valid species in a botanical sense of the term. Following the path of Sabouraud, particularly in the twenties of the present century, the strange habit developed among medical mycologists to create unneces-

[10] What we currently understand under "Sabouraud's media," the DIFCO Sabouraud media, for instance, has nothing in common with the original French media of Sabouraud but the name. The American DIFCO media use chemically pure carbohydrates and peptones and there is no identity whatsoever of the same species on the original French and DIFCO Sabouraud mediums.

[11] Bruhns and Alexander (Allgemeine Mykologie und Biologie, Jadassohn's Handb. d. Haut- u. Geschlechtskr. Xi, 1928, J. Springer, Berlin) give many comparative pictures of dermatophytons on these different media.

sarily, without valid botanical differences, a great number of new species in the genus *Microsporum*.

Beside the valid type species of the genus *Microsporum*, *M. Audouini*, *M. lanosum*, *M. equinum* and the far Eastern *M. ferrugineum*, Bruhns and Alexander (**10**) list not less than 25 different "species" of *Microsporum*. This "differentiation," of course, has no botanical validity. The reason of this erroneous trend in creating new species can be easily found in the fact that Sabouraud and after him many medical mycologists did not take into account the great, sometimes, accessive *variability* and the *dissociation* within the valid species of the dermatophytons. Emmons (**11**) gave a graphic demonstration of these facts in the genus *Achorion* (1932). Benedek (**12**) demonstrated particularly the phenomenon of dissociation in the genus *Microsporum* (1938).

I have to specifically mention two "satellites" of the *M. Audouini* on the basis of my large experience, which caused much unnecessary discussion in Europe. Guéguen in 1911, W. Fischer in 1921 described a *Microsporum depauperatum*, Klehmet in 1919 and in 1921 again presented a *Microsporum pertenue* as new species of the genus *Microsporum* belonging to the anthropophilic (human) group of *M. Audouini*.

I saw not so rarely among the hundreds of cultures of *M. Audouini* similar strains which fairly well compared in their rate of growth, color, surface configuration with the *M. depauperatum* or *M. pertenue*. Through comparative microscopical examination, the attitude of these deviating strains on rice medium, their biological attitude on rice in the presence of the unilateral stimulating symbiont of *B. weidmaniensis* Benedek (**13**), left no doubt that these species are invalid. In the best case, they are to be considered as slight variants of an unstable species (*M. Audouini*). Bruhns and Alexander also considered *M. depauperatum* and *M. pertenue* nothing more but as slight variants of *M. Audouini*.

It is of great historical interest to refer here to the thorough study of Ch. J. White on "Ringworm as it exists in Boston," in 1899. This great pioneer and excellent observer called attention

at this tender age [12] of medical mycology that "there were four anomalous cultures isolated from specimens of *M. Audouini.*" Yet, in recognizing the "variants," he did not create unnecessary new species. Ch. J. White's description of these four "anomalous cultures" deserves to be taken from oblivion, to show posterity the precise observation, evaluation and description of mycological phenomena by an early pioneer, which height was not reached by many later workers in this field.[13]

Based on our present knowledge and retaining in principle Sabouraud's system of the dermatophytons as the only adequate system for dermatologists, although botanically it may be objected, I recognize only one anthropophilic type of the genus *Microsporum*, the *M. Audouini.* Their satellites listed by Sabouraud, compiled by Bruhns and Alexander, have to be dropped entirely. It is commendable that Lewis and Hopper

[12] Sabouraud's study on dermatomycoses as a preliminary report was published in book form in 1894. (Sabouraud, R.: Les Trichophyties Humaines. Paris. Rueffs & Co., 1894.)

[13] "There were four anomalous cultures isolated from specimens of *M. Audouini.* The first plant was evidently closely allied to the usual type, for the growth consisted of the white aerial, fluffy hyphae, but the central tuft was displaced by an area perhaps 1.5 cm. in diameter, much denser and whiter than the surrounding growth, and the usual concentric circles and the radiating sulci were totally lacking."

"The second important variation exhibited a growth whose vitality seemed to be greatly diminished. The usual abundant growth of the hyphae occurred about the inoculated tissue and then all further life apparently ceased. This phenomena was observed in 3 cases—2 from the scalp and one from the neck."

"The third of the rare forms of culture was derived from the beard of a man of 45. The growth is composed fundamentally of very delicate radiating hyphae, laying in such a transparent layer that they produce no color, but assume that of the medium. Superimposed is a homogeneous, downy, white layer, which is not produced as quickly as that of the hyphae, but increases peripherally, leaving always a free margin of the colorless hyphae."

"The fourth anomaly was extremely interesting and one wonders if the microsporum causing this culture can be related to the Italian plants which produce the violet cultures described by Mibelli. This culture was derived from scales from the scalp of a child of four. The central portion consists of a rosette-like elevation so dark that one would hesitate to describe its color. Surrounding this central zone is a circle composed of delicate, radiating hyphae, of a red-violet tint, and outside of all a third belt of pale lavender. The growth is distinctly small and never attained a diameter of more than 1.5 cm." —(This latter variety, of course, definitely refers to *Tricophyton violaceum.*)

already carried out this logical simplification and dropped all "satellites" of *M. Audouini* as separate species.

As to the zoöphilic type of *Microsporum*, the second most frequent species in my material was the *M. lanosum* (Sabouraud, 1907). I recognize only this species as valid and I drop the *M. felineum* (C. Fox and Blaxall, 1896) as a separate species entirely. Jacobson (**14**) listed *M. felineum* only as a "satellite" of the *M. lanosum* in 1932, Lewis and Hopper (1939) dropped it even as such. Davidson and Gregory (**3**) followed Langeron and Milochevitch (Ann. Parasit. **8**: 465–505, 1930) in joining *M. lanosum* Sab. 1907 with *M. felineum*.[14]

In my own experience I could not find any support for the purpose to carry on *M. felineum* either as a satellite or, let alone, as a species. As to the clinical picture they cause the same type of lesions. As to their microscopical morphology in the lesion, in the culture, as to their attitude on natural mediums (rice) except for some slight difference in the giant culture (varying from medium to medium) there are no valid differences between the two.

To keep up even in name the differentiation between *M. caninum* and *M. felineum* would seem to me all the more difficult and without any real factual basis, because one cannot prove the transmission of the infecting agents from the respective animals (dog, cat) to humans. Despite strenuous search and questioning, even inspecting the pets, dogs and cats, from respective households, clinically and under Woodlight, I could not connect in my material any case with *M. lanosum* or *M. felineum* to dogs or cats in the surroundings of our patients.

It is possible that zoöphilic species are originally bound to their animal hosts alone or mainly. Later in the course of cross infections between animals to humans and vice versa,[15] however, the zoöphilic species were carried on so persistently by human hosts

[14] Sabouraid himself wrote: "En fait, rien ne savait différéncier mycologiquement le *Microsporum felineum* du *Microsporum lanosum;* l'examen microscopique de leurs cultures les montre indifférenciables entre elles." Les Teignes, p. 687. Paris, Masson & Co. 1910.

[15] Davidson and Gregory (**3**) reported a fine observation of cross infection between kitten and child and vice versa.

alone that there was no necessity for the pathogenic agent to return to the primary animal host.

This change in the host for the originally zoöphilic *M. lanosum* is proved also by the present material. *M. lanosum* caused, at least in the half of the cases related to it, torpid, non-inflammatory lesions on the scalp, indistinguishable from the anthropophilic *M. Audouini* infection. On the other hand, the evidence showed that half of the kerions was caused by *M. Audouini*, a fungus causing originally only torpid, non-inflammatory lesions on human scalps.

I have to call attention to this conspicuous change in the host relationship of zoöphilic and anthropophilic species, because the earlier accepted rule that anthropophilic species in humans cause only non-inflammatory, zoöphilic species, on the contrary, only inflammatory lesions, does not hold true at present any more.

In the genus *Trichophyton* the whole material revealed only one species, *T. crateriforme*, once, in two White siblings.

Morris Moore (**15**) stated in a discussion of the paper of Livingood and Pillsbury, in very general terms, that in the past several years, he found only two cases of tinea capitis among his material due to *M. Audouini*. The rest were due to *M. lanosum*. He found "a number of strains of this particular organism (*M. Audouini*) so closely related to *lanosum* that he doubts if it is possible to distinguish between the two."

This comment is rather peculiar. It is true that in a large material like that of Ch. J. White, mine, one will find a number of strains being peculiar, but they are under no circumstances "related to *lanosum*." To raise doubt, however, as M. Moore does about their being distinct species (*M. Audouini* and *M. lanosum*) would mean, without any positive evidence advanced, to carry confusion in well established knowledge. Moreover, Conant first showed conclusively that using polished rice medium any strains—peculiar or not—of *M. Audouini* can be distinguished from any strain of *M. lanosum*. Benedek (**13**) substantiated Conant's observation completely.

A further remark of M. Moore that tinea capitis in the Middle West region was found to be largely due to *M. lanosum*, does not hold true, either. Chicago is uncontestably in the center of the

Middle West. The present material reveals 79 per cent *M. Audouini*, and only 13.9 per cent *M. lanosum*. Epidemiological research must always be based on large material observed and collected through a longer period of time to enable us to make proper generalizations.

Livingood (**16**) pointed out in his closing remarks that they had "no difficulty" in distinguishing the species of fungi by the method outlined in their paper and it is well to emphasize again that the examination of the giant culture under Wood's light is an extremely valuable aid in identification."

I shall come back later to this last remark. Eventually, I have to make a few remarks concerning *Achorion gypseum* Bodin, 1907. I identified this fungus three times in my material. According to the statistics of Bruhns and Alexander (1928) this fungus is one of the rarer pathogenic agents. It was found in scattered single cases in France (Sabouraud, 1894, Bodin, 1907); in Italy (Porcelli, 1922, Mazzini, 1925, Truffi, 1913 and 1923); in Austria (Vienna) (Scherber, 1914, Stein, 1921, Tschirntsch, 1927); in Denmark (Rasch, 1922); in Germany (Bruhns and Alexander in Berlin, Werther in Dresden); in Hungary (Neuber, 1921).

As far as I could ascertain *Achorion gypseum* was found and reported in the United States previously only twice, once by Rockwood (**17**) in Boston, and another time by Emmons (**11**) in New York. This is, however, the first time that occurrence of *Achorion gypseum* is reported from Chicago.

The taxonomic position of this fungus is most peculiar. Sabouraud in 1894 (**18**) described it as a *Trichophyton* (Trichophyton du chien); Bodin, in 1907, (**19**) as an *Achorion*; Conant, in 1926, (**20**) as a *Microsporum*. Lewis and Hopper, in 1939, gave some short and incomplete accounts of this fungus making the terms: *Microsporum fulvum*, *Microsporum gypseum* and *Achorion gypseum* in an unmistakable effort of simplification of our nomenclature, synonymous (l.c.p. 284).[16] This is, however, an obvious error.

[16] Lewis and Hopper registered, in 1935–1938, in New York three times "*Microsporum fulvum.*" Livingood and Pillsbury, in 1941, found "*Microporum fulvum*" once in Philadelphia. They attached a footnote (l.c.p. 45) to *M. fulvum:* "Mycologic studies done in collaboration with the Laboratory of Dermatological Research, University of Pennsylvania." By implication,

The type species of *M. fulvum* (J. Uriburu, 1907) was established by Sabouraud in a specimen sent to him by Uriburu from Buenos Aires. Ballagi (**21**) found this fungus in 1916 in three cases in Hungary. Sabouraud, Ballagi emphatically pointed out that the microscopical characteristics of this fungus in the hair, in other words, the relationship between fungus and hair shaft is absolutely *identical with that of M. Audouini. M. fulvum is definitely a microsporum.*

On the other hand, considering the relationship between the fungal elements of *Achorion gypseum* and the hair, I found a surprising variation in this respect. In one case, it revealed the type characteristics of a *Trichophyton endothrix;* in the second instance it showed the type characteristics of a *Trichophyton ectothrix* and in the third case it was a typical endo-ectothrix. It represents one of the fastest growing species of the dermatophytes with a surabundance of organs of fructification, multilocular giant macroconidia (spindles), some aleurospores. A 72 hours old hanging drop culture shows already a surprisingly great number of these spindles.

The only valid species name of this fungus is therefore *Achorion gypseum* Bodin, 1907; it must not be confounded with another valid species, the *Microsporum fulvum*, and, of course, they are not identical and their names are not synonymous.

In Sabouraud's complicated system of dermatophytes the first basic principle is the relationship of the fungous elements to the hair. In this respect the *Achorion gypseum* is a *typical Trichophyton* as Sabouraud first classified it in 1894.[17] Whether it is

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I may assume that Fred D. Weidman identified this fungus as *M. fulvum.* Therefore, I accept this diagnosis, although Livingood and Pillsbury do not give either an illustration or description about the characteristics of this fungus in its relationship to the hair.

[17] It is interesting that Sabouraud changed his views in the following decades. But seemingly, except for his first case (1894), he had little or no opportunity at all to carry on a more thorough study of *Achorion gypseum* and its relationship to the hair. This fungus does not occur in Paris, or at least after its discovery by Sabouraud in Paris, in 1894, and after its rediscovery and final valid description by Bodin, 1907, it was never mentioned again in the French literature as far as I could ascertain.

Sabouraud, in 1936, writing the last time on this subject (R. Sabouraud: Les Microsporums, in Nouvelle Pratique Dermatologique, Masson & Co.,

an endothrix, an ectothrix or ecto-endothrix, the mycelium is straight, breaking up in rosary like *rectilinear* rows, as the case is in *Trichophyton*. No hair—I examined dozens of them in each of the three cases—ever revealed a spore sheath consisting of *spores of mosaic-like arrangement* which is distinctive and absolutely characteristic for the relationship between parasite and hair in the whole genus of *Microsporum* without exception.

The second of the basic principles, if available, is the relationship of the infecting fungus to the hair in experimental animals, most suitable in guinea pig. All the three strains of *Achorion gypseum* showed in guinea pig hair a typical, purely endothrix character and not a mosaic-like spore sheath, so characteristic for *M. lanosum* in guinea pig hair, for instance.

The only group of dermatophytons characterized by permanent or casual scutulum formation is the genus *Achorion*. Bodin first observed this scutulum formation by *Achorion gypseum* and therefore he put this fungus into the genus *Achorion*. The *Achorion Quinckeanum*, for instance, is placed in this genus for the same reason, the scutulum formation, although it has a typical *Trichophyton* fructification.

Conant (**20**)—and lately Sabouraud [18]—put *Achorion gypseum* as "*Microsporum*" *gypseum*, in the genus *Microsporum* on account of its surabundant production of spindles as organs of fructification. That is, however, no valid reason. Even though the genus *Microsporum* is mainly characterized by the production of macroconidia as the main organ of fructification, we meet with macroconidia in the genus *Epidermophyton* and even exceptionally in the genus *Trichophyton*.[19] Yet, nobody would try to put *Epidermophyton inguinale* in the genus *Microsporum* due to its characteristic organ of fructification, the macroconidia. The

Paris, 1936, vol. 11: 125 pp.) made the following statement: (pl. 147, l.c.) "J'ajoute que l'*Achorion gypseum* de Bodin est mycologiquement pareil aux Microsporums animaux et de toute evidence doit, du point de vue botanique, être rangé à côté d'eux. La seule difference notable entre eux vient de ce fait que les fuseaux, en navettes, de ces parasites sont réunis on cymes de dix et douze fuseaux, à l'extrémité d'un même ramau aérien."

[18] See footnote page 31.

[19] For instance, in *T. purpureum*, *T. gypseum*, cf. A. E. Edgecombe: *Trichophyton purpureum* (Bang) and *Trichophyton gypseum* (Bodin). Arch. Dermat. & Syph. **46**: 651–660. 1942.

determining factor for the latter group of fungi is their relationship to the stratum cornum, and as a negative characteristic that they never invade the hair shaft.

Eventually the cultural characteristics, the rate of growth, the early, rapid and abundant production of spindles, the flat giant culture with its fawn color, last, but not least, its accessive variability on artificial media (Emmons, l.c.) definitely separate this dermatophyton from the genus *Microsporum*.

Lewis and Hopper proved it conclusively (l.c.p. 250, *fig. 51*) by the illustration given for "*Microsporum fulvum*" that they had *Achorion gypseum* and not *Microsporum fulvum* in their hand. The same holds true for the case published by Moore and Conrad (**22**).[20]

5. IDENTIFICATION OF THE GROWTH AND DETERMINATION OF THE SPECIES

For this purpose, I used (1) the observation of test-tube cultures (Difco Sabouraud glucose or maltose medium) directly on the microscope stage by means of Benedek's mycological stage clamps; (2) hanging drop cultures by using thin films of the same medium; (3) polished rice medium; (4) the fluorescence phenomenon of test-tube cultures and giant cultures.

(1) The direct observation of the development of the test-tube cultures under the microscope, made possible as a routine method by Benedek's mycological stage clamps, has great advantages, particularly in a mass investigation as the present one. The special technic of inoculation of agar slant mediums, the purpose, the necessity and advantages of this method for routine examination, for comparative study and for photomicrographic purposes were amply described in several publications by the writer. Here, I simply refer to them (**23**, **24**, **25**).

(2) As to the hanging drop cultures I have only to remark that I usually used thin films of Difco Sabouraud glucose or maltose

[20] Moore and Conrad illustrated an infected hair demonstrating "filaments and *chains of spores*." Moreover, they did not give any sufficient description of their own observation of the basic relationship between fungus and hair. Their only sentence (p. 613): "Microscopically the fungus on the infected hair may simulate microsporum of animal origin or at times *Achorión Schoenleini*" is strikingly contradicted and disproved by their illustration given.

mediums as "hanging drop" instead of glucose or maltose broth. I always reached the same goal, even better, and the thin solidifying film was easier to handle than the broth. However, I often added 0.1 per cent eosin or 0.01 per cent methyl blue to the bulk of the mediums which caused vital staining of the whole growth giving beautiful detailed pictures during the whole development of the fungus. For more detail on vital staining of fungi, I refer to the publication of Williams (**26**).

(3) In my technic, however, the polished rice medium played the most important role for identification for anthropophilic and zoöphilic strains of *Microsporum*. As a rule every freshly isolated strain of *Microsporum* went through rice medium in the process of identification in the laboratory because this method is at present the only sure way to exactly differentiate anthropophilic and zoöphilic strains in the genus *Microsporum*.

During the comparative study of the genus *Microsporum* Conant (**20**) found that *Microsporum Audouini*, in sharp distinction from *Microsporum lanosum*, does not vegetate on rice medium. A few years ago already, during a study of a newly discovered species of *Microsporum*, *M. Stilliansi* Benedek, 1938 (**12**), making comparative investigations with *M. Audouini* and several zoöphilic species of *Microsporum* on rice medium, Benedek readily substantiated the observation of Conant as to the difference of viability of human and animal species of *Microsporum* on rice.

The present large study gave ample opportunity to prove again and again the correctness of this observation.

The use of polished rice medium eliminates the difficulties in identification of strains of *Microsporum* and it is the solution of the problems M. Moore referred to.

(4) Due to a remark of Livingood and Pillsbury (l.c.p. 54) that "Fluorescent light examination of giant cultures proved an extremely useful adjunct to standard methods of identification of fungus species," I was extremely intrigued, to pay special attention also to this method which in the hand of this writer in previous unpublished experiments did not lead to any useful and conclusive results. I was, however, not only intrigued by Livingood and Pillsbury's remark, I was directly struck by the lack of evidence

and by the want of any necessary reference to the work done in this field by others in due support of their statement.

Summarily, to begin with, there is no support for Livingood and Pillsbury's statement in the whole world literature as far as I could ascertain by careful search.

Authors who paid their special attention to the fluorescence phenomenon of dermatophytons in giant cultures are Berde (**6**) Davidson and Gregory (**27**), Mallinckrodt-Haupt and Carrié (**28**).

A few short remarks on fluorescence of hyphomycetes in general in giant cultures can be found before these authors in literature. Danckwortt (**29**) stated that colonies of molds fluoresce pale purple. Margarot and Devèze (**30**) made the same statement on *Trichophyton*.

Berde made his investigations into the fluorescence phenomenon of giant cultures of dermatophytons by using four different agar mediums with maltose, glucose, honey and a pepton agar medium free from carbohydrates. The fungi grown on these media could be divided according to their fluorescence into three groups. (1) *Hemispora porcelli* and *Hemispora rugosa*, *Microsporum ferrugineum*, *Trichophyton plicatile*, *Achorion Schoenleini*, *Saccharomyces Binot* and *Saccharomyces San Felice*, *Actinomyces bovis*, *Actinomyces madurae*, and the pigment producing strains of *Sporotrichum* do NOT fluoresce at all; their colonies appear in Woodlight dark gray or black. (2) *Microsporum Audouini; Trichophyton cerebriforme, violaceum, niveum, fumatum, equinum; Epidermophyton inguinale; Achorion Quinckeanum, Achorion gallinae* reveal a pale purplish fluorescence, while (3) *Oidium albicans*, Sporotrichums producing no pigment, *Mycoderma cutaneum* and the giant colonies of some yeasts showed up in a striking white light.

Berde had already observed, however, that the chemical compounds of the nutrient mediums play a decisive role in some dermatophytons as to the fluorescence of their growth on these mediums. He found, for instance, that *T. cerebriforme* fluoresces brown, grown on mediums containing carbohydrates, while it shows up in purple color on a carbohydrate-free medium. *Trichophyton violaceum* fluoresces purple with, and black without, carbohydrate in the medium. *Achorion gallinae* fluoresces purple

on maltose agar, red on carbohydrate-free medium. *Trichophyton gypseum asteroides* revealed a quite peculiar fluorescence, entirely different from any other, revealing a pale blue color, which was, however, inconstant as to its appearance and its distribution.

Berde came to the conclusion based on his extensive experimental work that fluorescence as an adjunct means for identification of dermatophytons can be used only for *T. gypseum asteroides under exclusion of all other fungi.*

Mallinckrodt-Haupt and Carrié have not been successful by examining 200 giant cultures of dermatophytons grown on Sabouraud agar mediums to establish a fluorescence in Woodlight corresponding to the clinical findings. Also numerous investigations on liquid Sabouraud mediums did not lead to any positive results. *By no means was it possible*, they concluded, either on Sabouraud's solid mediums or on liquid mediums with addition of different ingredients *to elicit a definitely proven fluorescence in a fungal culture.*

Davidson and Gregory noted that the green fluorescence of microsporum infected hairs is not observed macroscopiclally in cultures of the organisms when grown on Sabouraud's medium. The cultures frequently appear in a violet color under the Woodlight. Once a beautiful golden brown fluorescence has been observed from a culture of *M. felineum.*

I restricted my own experiment and observations to the commonest dermatophytons, *M. Audouini*, *M. lanosum*, using for comparison *Achorion gypseum*, *T. purpureum*, *T. violaceum*, and *Epidermophyton interdigitale.*

These few examples should reveal how unreliable the phenomenon of fluorescence is for the determination of species of dermatophytons in the culture.

The fluorescence is first a function of the nutrient mediums. It very probably depends also on the metabolic products elaborated from the changing chemical ingredients by the fungus itself.

M. Audouini fluoresced in peach-purple, dark-brown, fawn-brown colors changing with the mediums; different strains of this fungus often revealed changing fluorescence on the same medium.

TABLE I

ASPECT OF GIANT CULTURES OF SOME DERMATOPHYTONS IN WOODLIGHT

Species	Surface of the growth	Bottom of the media	Nutrient medium used	Age of the culture
M. Audouini No. 152	Peach-purple in the center, dark brown in the periphery	canary-yellow	D S M [1]	9 months
M. Audouini No. 137	Fawn-brown uniformly	canary-yellow	D S Gl [2]	3 months
M. lanosum No. 175	Chalky-white in center, green with deep purple-violet in the periphery	olive-green	O S M [3]	11 days
M. lanosum No. 171	Purple-violet uniformly	olive-green	O S M [3]	11 days
M. lanosum No. 168	Peach-purple uniformly	canary-yellow	D S Gl [2]	11 days
Achorion gypseum No. 145	White, covered up with deep purple-violet	olive-green	O S M [3]	11 days
T. violac.	Greenish-brown	olive-green	O S M [3]	6 months
T. purpureum	Peach-purple	olive-green	D S Gl [2]	7 months
T. purpur.	Purple	green	O S Gl [4]	1 year
Ep. interdigitale	Purple	green	D S Gl [2]	1 year

[1] Difco Sabouraud maltose.
[2] Difco Sabouraud glucose.
[3] Original (French) Sabouraud maltose.
[4] Original (French) Sabouraud glucose.

M. lanosum showed the same uncertainty and unreliability. On original (French) Sabouraud maltose agar the color of fluorescence changed from chalky-white to purple-violet, showing a tender peach-purple color on Difco Sabouraud glucose agar.

Trichophyton purpureum and *Epidermophyton interdigitale* gave the same purple fluorescence.

We deal with so many variables in the reaction of a fungus growth on any medium, artificial or otherwise, to filtered ultraviolet light (Woodlight) that *at the present time* I cannot but emphasize the *practical uselessness* of the fluorescence phenomenon for determination of species of dermatophytons. In this respect I cannot but substantiate the observations and conclusions of Berde, Mallinckrodt-Haupt and Carrié, Davidson and Gregory.

Livingood and Pillsbury might have found some solution of this complex and complicated problem. Yet, if so, they retained

all the useful information on their own technic which would have enabled the writer and other research workers to substantiate their results. Until such a time we had better consider their statement on the "extreme usefulness of fluorescence light examination of giant cultures for the identification of fungus species" as unwarranted and unsupported by any evidence.

To close the discussion on the diagnostic value of fluorescent phenomenon in tineal infections of the scalp I have to make a few remarks to a statement of Kinnear (**31**). In his experience *M. Audouini* infected hairs retained their fluorescence under Woodlight indefinitely mounted in liquor potassiae. Davidson and Gregory (**27**) already contradicted Kinnear's statement. They found that the extraction of the fluorescent substance may readily be demonstrated microscopically for single hairs mounting them dry on a slide and running a drop of 7 per cent potassium hydroxide under the coverslip. The hairs were examined under ultraviolet light. Almost at once the fluorescence in the hair began to fade, while the liquid in which the hair was mounted takes up the fluorescence.

I tried to duplicate both Kinnear's and Davidson and Gregory's experiments. I used 10 per cent and 20 per cent solution of potassium hydroxide (Kinnear did not state the concentration used) and the chlorallactophenol formula 1 and 2. I put single fluorescing hair in a drop of these solutions on a slide covered with a cover-glass. Then the fluorescence was controlled hour by hour for three hours and after 24 hours.

Potassium hydroxide destroyed the fluorescent principle in the hair within three hours, while the intensity of the fluorescence faded out little by little according to the observation of Davidson and Gregory and contrary to the experience of Kinnear. In chlorallactophenol the fading out of the fluorescence of the hair could be seen up to 5 to 6 hours. After 24 hours no fluorescence could be ever seen in these wet mounts.

Dry mounts (1, loc. cit. p. 192), hairs enclosed between two slides and framed with lantern slide tape,—however, preserve the fluorescence of tineal hairs indefinitely. They are rather welcome objects of demonstration at any time if occasion arises.

SUMMARY

The existence of CARRIERS of the pathogenic agents of tinea capitis is discussed, their number and importance in epidemiology are emphasized.

The use of Woodlight is advocated in detecting the carriers, in establishing the extent of the infection of the scalp, in the control of the progress and efficiency of treatment and in the final determination of a cure.

For the demonstration of fungi on direct microscopic examination the general use of chlorallactophenol formulas are recommended.

The taxonomic individuality of *Achorion gypseum* Bodin, 1907, is upheld in refutation of certain tendencies to make this species synonymous with *Microsporum fulvum* Uriburu, Sabouraud, 1910.

Finally, the availability of Woodlight for the purpose of identification of fungus growth in giant cultures is taken under scrutiny. The unwarranted statement of Livingood and Pillsbury about the extreme usefulness of this physical agent in identification of fungus species is refuted by the experience of other research workers and by the own experiments of the writer.

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REPRESENTATIVES OF THE MESOPHELLIACEAE IN NORTH AMERICA[1]

S. M. ZELLER

(WITH 6 FIGURES)

Abstoma reticulatum Cunningham was previously reported from California.[2] Recently Dr. W. H. Long has sent a specimen of a new *Abstoma* from New Mexico and there is also presented here a new genus, all of the three species of which are from far western states. This paper accordingly brings together descriptions of all of these representatives of the new family, *Mesophelliaceae*, as they occur in North America.

1. ABSTOMA RETICULATUM Cunningham, Linn. Soc. New So. Wales Proc. **52**: 242–243. 1927.

Fructifications depressed-globose, up to 6 cm. broad; peridium of two layers, exoperidium very thin or up to 2 mm. thick, fragile, fugacious, whitish or brownish, composed of hyphae intermixed with particles of earth or sand, sometimes merely a meshy hyphal layer covering the endoperidium, which is smooth, dark-colored, mostly umber or sepia-brown, tough, membranous, dehiscing by irregular rupture; gleba at first yellowish, then olivaceous with purplish tints, powdery; capillitium tinted, thin-walled; spores globose, 8–12 μ in diam., dark brown, distinctly reticulate.

Under or on duff under cypress or fir, central and northern California. January to July.

Specimens examined: California; Monterey county, Point Lobos, near Pacific Grove, *Gertrude S. Burlingham*, *7*, January 6, 21 and 22, March 26, 1937; Siskiyou county, near Horse Camp on Mt. Shasta, *Wm. B. Cooke*, *14642*, July 8, 1940.

The specimens from Mt. Shasta are referred here with some hesitancy because they are quite young.

[1] Published as Technical Paper No. 439, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

[2] Zeller, S. M. Further notes on fungi. Mycologia **33**: 196–214. 1941. (See p. 213.)

2. **Abstoma Longii** sp. nov.

Fructificationes depresso-globosae, obscure brunneae, 2 cm. crassae; exoperidio tenuo terroso, difficiliter separabilis; endoperidio membranaceo, sed duro et rigido; gleba primo molli dein obscure brunnea, floccoso-pulverulenta; capillitio tincto vel hyalino, flaccido; sporis globosis, obscure brunneis, echinulatis, 26.2–28.75 μ (cum aculeis); aculeis 3.7–5 μ longis, mollis, flexuosis, confertis.

Fructifications depressed globose, dark brown, up to 2 cm. in diam.; exoperidium thin with sand inclusions, mostly persisting; endoperidium papery thin, but hard and shell-like; gleba at first soft, then dark-sepia and flocculent, powdery; capillitium tinted or almost hyaline, threads short, fragmentary, flexuous; spores spherical, dark-brown, echinulate, 26.2–28.75 μ including echinulae, which are 3.7–5 μ long, close together, softish or flexuous.

In open sandy mesquite dunes, Jornado Range Experiment Station, about 23 miles from Las Cruces, Dona Ana county, New Mexico, *W. H. Long, 8310*, Nov. 11, 1918, **type.**

Radiigera gen. nov.

Fructificationes hypogeae vel epigeae, subglobosae; peridio duvel triplici; basis sterilis pulvinatis; capillitium fasciatans ad columellam basalam spongiosam percursum radians; gleba primo albida, molli, succoso-subcarnosa, postremo obscure brunnea vel atra, pulverulenta; basidiis subglobosis. Vel pyriformis, fasciatis, pendere ex radiant hyphae, quadri- vel multisporis; sporis obscure brunneis, sphaericis, verrucosis vel echinulatis.

Fructifications hypogeous or epigeous, subglobose; peridium of 2–3 layers; thickened sterile base supporting a spongy pulvinate to subspherical columella or core from which the capillitium fibrils radiate to the endoperidium; gleba at first white, soft, succulent to subfleshy, becoming black or dark-brown and powdery at maturity; basidia pyriform to subglobose borne in groups along radiating hyphae, 4–many-spored; spores colored, spherical, verrucose to echinulate.

The type species is *Radiigera fuscogleba* Zeller.

The genus *Radiigera* (*to bear or exhibit radii*) in the Gasteromycetes is essentially the counterpart of some of the higher Plectascales, such as *Elaphomyces*. *R. atrogleba* especially reminds one of *Elaphomyces* both externally and internally; externally because of the matrix-like or mycelial-spawn-like coating, and internally because of the black, powdery gleba with cottony

or evanescent core or columella. The spores are also similar to those of species of *Elaphomyces*.

The gelatinous nature of the endoperidium in fresh specimens sets the genus off from other related genera except *Mesophellia* of which Cunningham [3] says "The endoperidium is . . . composed of a pseudoparenchyma of closely woven, partly gelatinized hyphae."

In *Mesophellia* the central core of the gleba is compact and cheesy in consistency and is held in a central position by strands of the same tissue which extend to and are firmly attached to the inner wall of the endoperidium. In *Radiigera*, the core is essentially a subspherical columella which usually crowns the apex of the sterile base. In *R. atrogleba*, although attached at first, this core may become free or entirely collapsed or evanescent. In all three species the columella (core) is very delicate, either cottony or a soft pithy parenchyma.

Radiigera is similar to *Abstoma* in several respects. There is no apical mouth in the endoperidium, dehiscence occurring through the gradual weathering or breaking up of these membranes. The capillitium is unbranched and the spores are spherical.

The structure of the gleba in *Radiigera* is very similar to that of *Geastrum*. The gleba outside of the conspicuous columella or basal core is composed essentially of radiating fascicles of hyphae and capillitium arranged to simulate tubes which reach from the columella to the endoperidium. These fascicles are attached at both ends but in *R. fuscogleba* they separate readily from the endoperidium while in *R. atrogleba* they may separate from both, the columella and the endoperidium. Sections of young dried fructifications, cut tangentially across the fascicles in tube-like arrangement, reveal nothing which could be construed as true tramal tissues. The origin and development of the radial fascicles must be learned through the study of histological preparations of very young fructifications, however, before exact interpretations may be made.

Basidia are borne in groups along the hyphae which are included in the radiating glebal fascicles. Each group of basidia may originate more or less directly from the radial hyphae or are

[3] Loc. cit. p. 313–314.

borne on short hyphal branches. This arrangement of basidia is very similar to that illustrated by Fischer [4] for the basidia of *Podaxis*. The basidia of *Radiigera* are broadly pyriform with several spores as found in some other genera like *Calostoma*, *Sphaerobolus*, or *Lycogalopsis*. The spores are sterigmate.

The capillitium is long and mostly unbranched. In *R. fuscogleba* the filaments are variable, dark, flexuous, walls uneven, sometimes almost moniliform. In *R. atrogleba* the capillitial filaments are smooth and almost glistening, somewhat wavy, dark. In *R. Taylorii* there are two sizes of capillitial hyphae, which are only slightly colored.

To place a genus like *Radiigera* in its correct taxonomic position presents a perplexing problem because of its diversified affinities, and the lack of knowledge concerning the developmental morphology and the manner in which basidia are borne in some of the apparently affiliated genera. The simple course of basing the classification entirely upon the characters that may be seen in mature fructifications is misleading, but undoubtedly our present incomplete knowledge of the origin and morphology of the glebal tissues of many described Gasteromycetes will probably lead to imperfections in any systematic plan which may be chosen for these fungi.

In 1933 Fischer [5] devised a plan for the Gasteromycetes based on morphological development and the manner in which the basidia are borne. In this plan the genera which have apparent affinities with *Radiigera*, namely, *Abstoma*, *Castoreum*, *Geastrum*, and *Mesophellia*, have been referred to four distinct families, although *Castoreum* and *Mesophellia* were placed under "genera doubtfully included or imperfectly known."

Working independently at the same time and without Fischer's knowledge Cunningham [6] devised a plan which we believe more nearly satisfies this particular group of genera, especially *Abstoma*, *Castoreum*, *Mesophellia*, and *Radiigera*. His plan included

[4] Fischer, Ed. Gastromyceteae. E. & P. Nat.-Pfl. **7a**: p. 118. *fig. 91A* and *B*. 1933.

[5] Loc. cit.

[6] Cunningham, G. H. The Gasteromycetes of Australasia XV. The genera Mesophellia and Castoreum. Linn. Soc. New So. Wales Proc. **57**: 313–322. *illus*. 1932.

the tribe *Mesophellieae*, with tribes *Lycoperdeae* and *Geastreae* in the family *Lycoperdaceae*. It would seem, however, that Fischer's scheme for suborders and families is more feasible from the morphological viewpoint. It is proposed to combine the two ideas therefore and to include the *Mesophelliaceae* in the suborder *Lycoperdineae* (sensu Fischer). The *Mesophelliaceae* would therefore contain the genera *Abstoma*, *Castoreum*, *Mesophellia*, and *Radiigera*.

The systematic key will be as follows:

Suborder: LYCOPERDINEAE

I. Ectoperidium not opening stellately at maturity.
  A. Peridium 2-layered, dehiscing by an apical stoma (irregular or wanting in *Calbovista*, *Calvatia*, and *Mycenastrum*); capillitium attached or free, simple or freely branched.....1. LYCOPERDACEAE.
  B. Peridium 2–3-layered, indehiscent, or rupturing irregularly at apex; capillitium unbranched.....2. MESOPHELLIACEAE.

II. Ectoperidium opening stellately at maturity; peridium usually 4-layered, endoperidium dehiscing by an apical stoma; capillitium attached, unbranched.....3. GEASTRACEAE.

The genera in *Lycoperdaceae* and *Geastraceae* may be classified as in Fischer except the genera as included in the *Mesophelliaceae* below.

Mesophelliaceae fam. nov.

Peridium usually 3-layered, indehiscent or rupturing irregularly at the apex; capillitium unbranched; spores globose or ellipsoid, variously roughened or with a gelatinous sheath; basidia inflated, sterigmate.

KEY TO GENERA

Spores spherical, echinulate, reticulated or verrucose.
  Gleba without a sterile base.....1. *Abstoma*.
  Gleba with a sterile base.....2. *Radiigera*.
Spores ellipsoid, smooth or irregularly roughened.
  Gleba with a central core.....3. *Mesophellia*.
  Gleba without a central core.....4. *Castoreum*.

So far as known *Castoreum* and *Mesophellia* do not occur in North America.

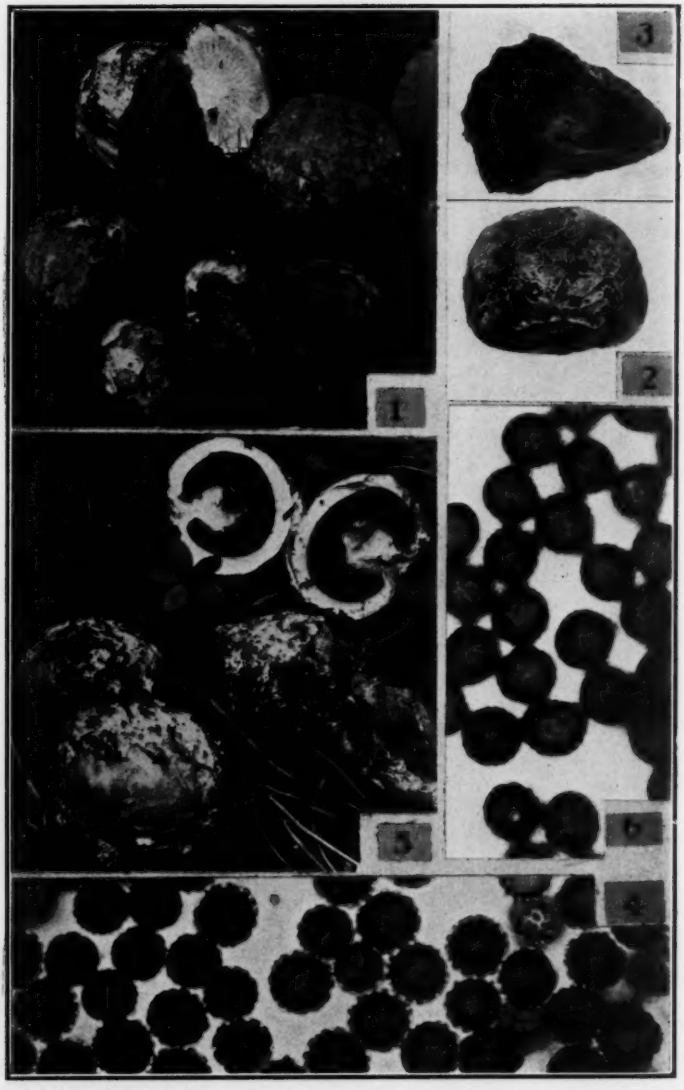

FIG. 1. Several fructification of *Radiigera fuscogleba* in natural habitat. Slightly reduced from kodachrome by Wm. B. Gruber. FIG. 2. Dried specimen from the type collection of *R. fuscogleba*. Note reticulate wrinkling of

1. **Radiigera fuscogleba** sp. nov.

Fructificationes subglobosae, 4–8 cm. crassae, basi saepe radicanti; superficie alba vel isabellina, saepe leniter purpurans, ligno-brunnea vel pallidior siccata, laevi, saepe innato-reticulato-fibrillosa; basis sterilis crasso-conica; columella subglobosa, mollo-spongiosa vel byssoidea, 5–8 mm. crassa; peridio tristratoso, facile a glebo separabili siccato; ectoperidio duplici, strato externo tenuo, filamentoso, strato interno (primo) subhyalino, parenchymato, 1 mm. crasso, tenuissimo siccato; endoperidio 2 mm. crasso, subgelatinoso, parenchymato, duro siccato; gleba alba, dein olivaceo-brunnea, funiculi hypham capillitiam que composita, ad columellam percursum radians; capillitium obscure, simplicis, flexuosum, inaequalum, 2.7–3.2 μ crassum; hyphis radiam subhyalinis, ramis brevis, basidiophoris; basidiis crasso-pyriformibus, 4–multisporis, brevi-sterigmatibus; sporis brunneis, globosis, alveolato-echinulatis, 4.7–5.2 μ, echinulae subtruncatae.

Sub foliis putridis in pinetis. Oregon, Amer. bor.

Fructifications subglobose 4–8 cm. in diam., with a distinct slightly projecting basal attachment; surface white to old gold or isabella color, sometimes with purplish tints, drying wood brown or lighter, smooth but sometimes with innate reticulate fibrils; sterile base broadly conic in vertical section, crowned by a subspherical columella which is soft, pithy toward the center and more cottony farther out, 5–8 mm. in diam.; peridium of 3 layers, not readily separating from each other but separating from gleba on drying; outer very thin layer (or surface pelt) filamentous, main ectoperidial layer of light-colored, large-celled parenchyma, about 1 mm. thick, drying to papery thinness; endoperidium of dark, large-celled and thin-walled parenchyma, somewhat gelatinous, 2 mm. thick, drying to thin, flinty hardness, inner surface a very thin filamentous layer of hyphae very similar to capillitium; gleba white throughout at first, drying olive brown, com-

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surface resulting from drying. These wrinkles follow more or less the reticulate fibrils over the surface. About natural size. FIG. 3. Vertical section of a fructification of *R. fuscogleba* in the type collection. Note sterile base crowned by a globular columella of two textures and tones, the central lighter colored and pithy, the outer zone slightly darker and more cottony. Note also the radiating fascicles from the columella to the endoperidium. About natural size. FIG. 4. Spores of *R. fuscogleba*. Note the alveolate-echinulate epispore. × 2000. Photograph by F. P. McWhorter. FIG. 5. Several fructifications of *R. atrogleba* in natural habitat. This shows three fructifications enveloped in a more or less common ectoperidium as described in the text. There is also a vertical section of a sporophore showing spherical columella, black gleba with radiating fascicles, and thick peridium, with more or less easily separable ectoperidium. Slightly reduced from kodachrome by Wm. B. Gruber. FIG. 6. Spores of *R. atrogleba*. × 2000. Photograph by F. P. McWhorter.

posed of radiating fascicles of hyphae and capillitium arranged to simulate tubes, fascicles attached to the columella and inner wall of endoperidium, readily separating from latter on drying; capillitium dark, unbranched, flexuous, 2.7–3.2 μ in diam., walls uneven, sometimes almost moniliform; fasicular hyphae small, almost hyaline, with short branches, all basidia-bearing; basidia broadly pyriform, 4–many-spored, with short sterigmata; spores dark, spherical, 4.7–5.2 μ, alveolate-echinulate, echinulae somewhat truncate, often connivent at their apices.

Hypogeous or epigeous in duff of coniferous forest, Mt. Scott, Portland, Multnomah county, Oregon, collected by *Wm. B. Gruber* (No. *8*), July 1943, **type**, and embedded in a thoroughly decayed log (ash?), Jefferson, Marion county, Oregon, *H. C. Gilbert*, Sept. 1929 (both in Zeller Herb.).

2. **Radiigera atrogleba** sp. nov.

Fructificationes depresso-globosae, infra umbilicatae, 3.5–5 cm. crassae, 2.5–3.8 cm. altae, exoperidium completum, confluendo stromatiforme individuorum complurium commune, crassum, compactum, coactile, ab endoperidio subfacile separum; superficie hebeti, inaequali, albi, canescenti, siscatate "tilleul-buff" vel "light vinaceous fawn" vel "buckthorn brown"; endoperidium 4–5 mm. crassum, albidum vel pallido-viridum, duplex, strato externo circa 1 mm. crasso, compacto prosenchymatibus, strato interno circa 3–4 mm. crasso subspongioso parenchymatibus; columella subglobosa, 10–15 mm. crassa, alba, canescenti, siccatate obscuriore, byssiodo-parenchymatam composita, saepe collabente, basi sterili pulvinati insedente; gleba atra, pulverascens; capillitio hyphisque basidiophoris columellae superficiee internae endoperidioque adnato, simplicio, laxo, obscuro, 2.5–3 μ crasso; hyphis basidiophoris hyalinis, ramis brevis, parvis; basidiis latopyriformibus, 4–multi-sporis; sporis obscure, subglobosis, verrucosis, 5.6–6.2 μ.

In terram arenosam subimmersum, prope McCall, Idaho, Amer. bor.

Fructifications depressed-globose, umbilicate under the slight sterile base, 3.5–5 cm. broad and about ¾ as high, several fructifications together may be embedded in a very conspicuous spawn-like mycelial mat, as well as a heavy felty, compact layer immediately surrounding the sporophores which also constitutes the exterior layer of the peridium (ectoperidium); ectoperidium surface felty, dull, rough, white to grayish at first, drying tilleul-buff to light vinaceous-fawn or even buckthorn brown, of fine somewhat nodose hyphae, more or less readily separating from the endoperidium which has an even, dull, felty surface with whitish or rosy tints when fresh (or changes to olivaceous tints, such as chamois or isabella color when bruised), drying pale pinkish buff;

endoperidium 4–5 mm. thick, pure white or with pale greenish tints, of two layers, outer about 1 mm. thick, of very compact, small-celled prosenchyma, the inner 3–4 mm. thick of large-celled parenchyma; columella subspherical, 10–15 mm. in diameter, arising from a slightly elevated sterile base, white to grayish, drying darker, of very pithy parenchyma, sometimes evanescent or almost totally collapsed; gleba black, composed of radiating plate-like fascicles of hyphae and capillitium arranged to simulate flattish tubes, fascicles attached to the columella and inner wall of the endoperidium, readily separating from both on drying; capillitium unbranched, smooth, almost glistening, somewhat wavy, dark, 2.5–3 μ in diam.; fasicular hyphae small, hyaline, with short branches, all basidia-bearing; basidia broadly pyriform, 4–many-spored; spores dark, subspherical, verrucose, often with a prominent sterigmatal scar, 5.6–6.2 μ in diam.

Hypogeous or epigeous in sandy soil of a creek bed among tamarack and white pine, near McCall, Valley county, Idaho. Collected by *Wm. B. Gruber* (No. *P–20*), Aug. 20, 1943, **type** (in Zeller Herb.).

Mr. Gruber has furnished the following field notes on *Radiigera atrogleba:* "The outer crust tough and hard like wood when dry. The black core (*gleba*) radiates from the center and from below. The black material consists of an ink-like substance, staining everything but it washes off easily with water. Odor metallic, resembling that of actual ink. The puff balls appear in closely connected, almost ingrown, clusters of from 15 to 30. They are deep in the soil and only a few of the balls are visible. When 'unearthed' the cluster of puffballs is found to be protected by a fragile, thin, mycelial mat which envelopes the whole cluster except the top part of those exposed to the surface of the ground. I saw the plant only in mature stages of development, however, and it appeared as though the mycelial spawn might have completely enveloped the balls in younger stages. Later stages of development show that the black juice changes to a dry spore powder."

The illustration of the spores (FIG. 6) shows the verrucose nature of the epispore as well as the sterigmatic scar in a few cases. At the base of the spore the verrucae radiate from the sterigmatic scar.

3. **Radiigera Taylorii** (Lloyd) Zeller, comb. nov.

Mesophellia Taylorii Lloyd, Myc. Notes **73**: 1305. *fig. 2914.* 1924.

Fructifications subglobose, 2–3 cm. in diam., with a distinct rooting basal scar; surface drying dull drab or lighter, even, smooth or slightly velutinate; sterile base white, pulvinate in vertical section, crowned by a subspherical, white, cottony-soft columella, sometimes detached from the base as a free core, sometimes almost evanescent; peridium of two closely adnate layers which become easily separable when dry; exoperidium eggshell thin when dry, cartilaginous; endoperidium white, gelatinous, about 3–4 mm. thick when fresh, drying very thin and light buff; gleba buffy-brown; capillitium in fascicles of long, parallel, non-branched filaments, fascicles extending radially from columella to endoperidium, composed of capillitial filaments and basidiophorous hyphae; capillitial filaments hyaline to slightly straw-colored, some large, up to 4 μ in diam., usual ones smaller, 2–2.5 μ in diam., apparently not branched; basidia borne in groups along the radiating hyphae, pyriform, up to 11- or 12-spored; spores sphaerical, slightly straw-colored to very light brown in mass, slightly verrucose, 2.5–3.75 μ.

Hypogeous under loose leaf mould of redwood near Eureka, Humboldt Co., California. Summer. The type was collected by *C. Wilder Taylor* (In Lloyd Collections, Smithsonian Inst., and in Zeller Herb.).

Under date of Sept. 30, 1924, the writer received the following note from Mr. C. G. Lloyd: "I enclose specimen that should be of interest to you. It will be published in the next issue of my notes, now in the printer's hands." He referred to a part of the type of *Mesophellia Taylorii* Lloyd. Mr. Lloyd was never satisfied with his reference of this species to the genus *Mesophellia*, according to his personal statement to the writer as well as to his comment on a later collection,[7] as follows: "Our American plant will no doubt be called a new genus in time," on the grounds of the soft, "cottony core, globose spores, and gelatinous endoperidium" . . . which are not to be found in other species of *Mesophellia*.

[7] Stevenson, J. A., and Edith K. Cash. The new fungus names proposed by C. G. Lloyd. Lloyd Library & Museum Bull. 35 (Mycological Ser. No. 8): 189. 1936.

Dodge,[8] who erroneously included *Mesophellia* in the Plectascales, excluded *M. Taylorii* from that genus on the same grounds as Lloyd had mentioned and further suggested its similarity to "juvenile, unexpanded stages of some puff-ball." Cunningham [9] believed "*M. Taylorii* was erected upon an unexpanded *Geaster*."

It differs, however, in several characters from *Geaster* and *Astraeus*, although it has some other characters in common with the former, and Mr. Lloyd, we believe, was too keen a student of *Geaster* to have made that mistake. There were several fructifications in each of the two collections received by Lloyd from California. None of them have an apical pore in the endoperidium, nor does the exoperidium divide regularly into astral rays. Both peridial layers break up irregularly. The fructifications accordingly remind one of *Abstoma*.

As soon as the writer received the specimens of *Radiigera fuscogleba* and *R. atrogleba* he was immediately reminded of their similarity to *Mesophellia Taylorii* Lloyd, and the three species have proved to possess likenesses enough to constitute a generic concept.

KEY TO SPECIES OF RADIIGERA

Spores echinulate, gleba brown.....1. *R. fuscogleba.*
Spores verrucose.
Spores 5.6–6.2 μ, exoperidium a heavy felt-like layer, gleba black at maturity.....2. *R. atrogleba.*
Spores 2.5–3.7 μ, exoperidium thin, cartilaginous when dry, gleba light brown or creamy.....3. *R. Taylorii.*

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[8] Dodge, C. W. The higher Plectascales. Ann. Myc. **27**: 156. 1929.
[9] Cunningham, G. H. Gasteromycetes of Australasia. XV. The genera *Mesophellia* and *Castoreum*. Linn. Soc. New So. Wales **57**: 313–322. *illus.* 1932.

BRAZILIAN CHYTRIDS IV. SPECIES OF ROZELLA

JOHN S. KARLING

(WITH 28 FIGURES)

Among the numerous saprophytic chytrids found by the author (**1944 a, b, c**) in water and soil cultures collected from various parts of the Amazon Valley in Brazil, occured six intramatrical species which parasitize the mycelium or sporangia of *Pythium*, *Achlya*, *Cladochytrium*, *Endochytrium*, *Rhizophlyctis*, and *Rhizophydium*. These parasites belong in the genus *Rozella* of the family Olpidiaceae, and five of them have been recorded previously from the United States. The sixth species, however, has not been found outside of Brazil and appears to be a new species. The discovery of these hyperparasites in South America indicates that the genus *Rozella* is widely distributed geographically as well as in host range.

At the present time species of this genus are segregated in two groups on the basis of whether or not the thallus divides into several segments which subsequently develop into sporangia or resting spores and become delimited by cross walls in the host cell. In the monosporangiate nonseptigenous group, the thallus forms one sporangium or resting spore and stimulates hypertrophy but not multiseptation of the host cell. The second group embraces the septigenous polysporangiate species in which the thallus is reported to cleave into segments which become delimited by the formation of host cell walls between them, mature in basipetal succession, and develop into sporangia or resting spores. So far thirteen monosporangiate and three polysporangiate species have been recorded in mycological literature. Of the species collected in Brazil, five belong in the former and one in the latter group.

NONSEPTIGENOUS MONOSPORANGIATE SPECIES

1. ROZELLA LAEVIS (FIGS. 1–19).

This species was previously found by the author (**1942 b**) as a parasite in *Pythium gracile* in Virginia and diagnosed as a new

species on the basis of its hyaline, smooth resting spores. In Brazil it occurred in an unidentified species of *Pythium* from a soil culture collected at Saõ Carlos, Matto Grosso. This host closely resembles *P. gracile*, but its identity has not been definitely established. The parasite also agrees so closely with the one described from Virginia that it is accordingly regarded as the same species. Inasmuch as *R. laevis* was diagnosed only briefly and not illustrated, a further account with figures of its structure and development is presented here. As is most species of *Rozella* its zoöspores are quite small, 1.5–1.8 × 2.9–3.3 μ, with a broad posterior and a tapering anterior end (FIG. 1). Occasionally, large abnormal, multiflagellate zoöspores (FIG. 3) are formed as the result of incomplete or unequal cleavage in the sporangium. The normal spores contain a dense globular body or area in the center which appears to be somewhat granular in structure, but is not highly refractive. As the zoöspores approach the end of the active swimming phase, they become oval and taper at the posterior end. Then as they come to rest they round up and become spherical in shape. If the quiescent zoöspore is in contact with a host cell (FIG. 4) it develops a short penetration tube which penetrates the wall. The content of the spore then flows slowly into the host cell as a naked plastic body and becomes more or less obscured by the host protoplasm. The empty spore case remains attached on the outside for a short time, but soon becomes wrinkled, collapses, and disappears as in *R. Cladochytrii* and *R. Rhizophlyctii* (Karling, **1942 a**). The newly-entered parasite appears to be closely enveloped by the host protoplasm, and no physical antagonism is visible between the two protoplasts. Within a few hours, however, the host hypha begins to enlarge in the region of infection, and if the parasite lies in or near the tip of a branch the latter usually becomes clavate in shape (FIGS. 5–8). As hypertrophy increases the protoplasm becomes denser and more coarsely granular in appearance (FIG. 5), so that the boundary parasite is obscured. With further enlargement of the host cell, however, numerous vacuoles begin to appear (FIG. 6) in the protoplasm, but as was pointed out by the writer (**1942 a**) in relation to infection by other species of *Rozella* it is not certain that all of them relate to the parasite. The vacuoles in the center

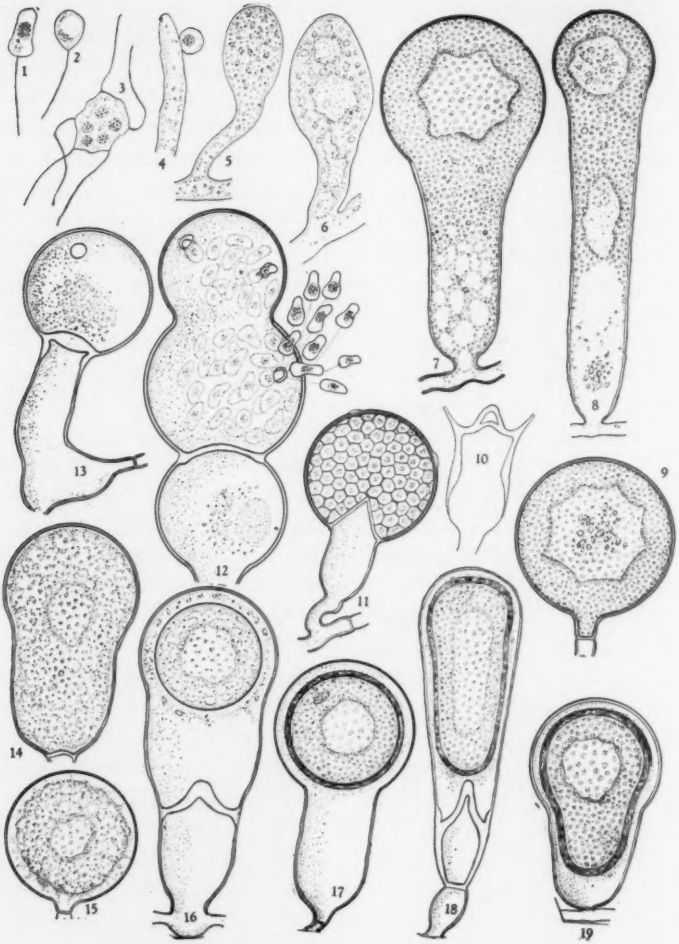
of the swelling later coalesce to form one or more larger ones as shown in figure 7. This figure shows a short hyphal branch which has been transformed into a large clavate swelling under the influence of the parasite. A large irregular vacuole is present in the dense upper part, while in the basal portion the protoplasm is relatively sparse and highly vacuolate. This condition becomes more pronounced as the protoplasm appears to move up and accumulates in the tip, until very little besides the primordial utricle remains in the base (FIG. 8). While these changes are taking place, a granular substance and variously-shaped globules, which look like extraneous waste material, accumulate in the vacuoles of the swollen portion (FIGS. 8, 9) and undergo Brownian movement.

The infected branches and intercalary portions of the host mycelium are usually delimited by cross walls from the rest of the thallus (FIGS. 9, 11, 13, 14, 15, 17, 18, 19), and in the event that such branches are fairly long an additional septum may be formed as the protoplasm accumulates in the tip (FIGS. 11, 12, 13, 16, 18). It is to be emphasized, however, that these septa do not delimit separate segments of the parasite as in cases of infection by septigenous polysporangiate species. The walls thus formed may be almost directly transverse, slight curved or concave-convex (FIG. 12), narrowly or broadly cone-shaped (FIGS. 11, 16, 18) and project upward into the more swollen portion of the branch. They may be comparatively thin (FIG. 11) or greatly thickened (FIGS. 10, 13, 16, 18). Oftentimes the longitudinal walls in the near vicinity of the transverse septa also become greatly thickened (FIGS. 10, 18). In some instances these thickenings were as pronounced as those produced by *R. Rhiphidii*, *R. Apodyae* and *Rozellopsis Waterhouseii* (Karling, **1942 a, c**, according to the figures of Cornu (**1872**) and Miss Waterhouse (**1940**). *Rozella laevis* thus induces septation of the infected parts and thickening of the walls in addition to causing hypertrophy. The hypertrophy, however, is localized and does not extend very far beyond the region of infection.

As was noted earlier, the *Rozella* parasite is difficult to see in the early developmental stages and may be completely obscured by the host protoplasm. This characteristic has been noted by

most workers on species of the genus, and has led several authors to believe that the protoplasm of the parasite is miscible with and infiltrates that of the host. While this condition may be suggested perhaps by figures 6, 7 and 8, the present writer, nevertheless, believes that the two protoplasts are distinct and immiscible. At maturity the parasite fills the hypertrophied portion which has been delimited by a cross wall, and its sporangium wall apparently becomes so closely applied to that of the host that the two are indistinguishable (FIGS. 9, 11). Figure 9 shows a relatively short branch which has been converted into a sphere and is completely filled by the parasite. The center is occupied by a large stellate vacuole in which lie numerous granules and extraneous material. At this stage of development low inconspicuous exit papilla are formed and project through the host cell wall (FIG. 11). The protoplasm then cleaves into zoöspore initials which mature and differentiate in the sporangium. When mature, they begin to move and glide over each other and among the dispersed extraneous granules or bodies. This movement increases in velocity and intensity until the zoöspores are rapidly swirling within the sporangium. The exit papillae then deliquesce and a small amount of hyaline slimy material oozes out and disperses in the surrounding water. Within one or two minutes the zoöspores begin to emerge in a dense stream which may extend for a considerable distance in the water before separation of the individuals occur. The zoöspores thus appear to be surrounded by or embedded in a slimy substance and lie quiescent for a few seconds. As this viscid material disperses they begin to jerk and wriggle about and soon dart away. After the zoöspores have been discharged a considerable amount of extraneous material in the form of granules, globular bodies and slimy substance remains in the sporangium. This material has the same appearance as that which was visible earlier (FIGS. 8, 9) in the large vacuoles, and apparently is waste material discarded by the parasite.

The resting spores of *R. laevis* occur after a period of abundant sporangium and zoöspore production. In their development the protoplasm in hypertrophied branches begins to contract from the host wall (FIGS. 14, 15) and develops a distinct wall (FIG. 16) of its own. The incipient spore thus formed is surrounded by a



FIGS. 1–19. *Rozella laevis*. 1, motile zoöspore; 2, zoöspore at end of motile period; 3, abnormal multiflagellate zoöspores; 4, infection of host mycelium; 5, beginning of hypertrophy; 6, later stage of vacuolate protoplasm; 7, large terminal clavate swelling with stellate vacuole at apex; protoplasm sparse and vacuolate in basal portion; 8, later developmental stage; 9, spherical swelling with large central vacuole in which lies extraneous material; 10, basal portion of a hypertrophied branch showing thickening of transverse and longitudinal walls; 11, delimited portion of terminal swelling filled with sporangium

narrow hyaline zone, and this in turn is enveloped by a layer of host protoplasm from which extend fine radial strands of cytoplasm. As the spore matures the wall thickens, while the protoplasm becomes coarsely and uniformly granular with a large vacuole in the center. The fully formed spores may be spherical (FIG. 17), oblong (FIG. 18), or somewhat obpyriform (FIG. 19) in shape and vary considerably in size. These characters are determined to a large extent by the size and shape of the swellings in which the spores occur. So far germination of the spores has not been observed.

As was pointed out in an earlier paper by the writer (**1942 b**) the resting spores of this species are similar in size to those of *R. cuculus* Butler (**1907**) which parasitizes *Pythium* sp., *P. intermedium* and *P. monospermum*. However, the spores of *R. laevis* are hyaline, while those of *R. cuculus* are brown to pale yellow in color. Although these species are very similar it is not certain that they are identical. Neither Dangeard (**1896**), Butler nor Tokunaga (**1933**) determined the size of the zoöspores of *R. cuculus*, and until this character is known the relationship of the two species will remain questionable.

2. ROZELLA CLADOCHYTRII.

This species was found parasitizing sporangia and the more tenuous parts of the rhizomycelium of *Cladochytrium replicatum* which had been collected at Saõ Carlos, Matto Grosso. Only a few diseased thalli were found, and the infection did not attain epidemic proportions. However, enough sporangia, zoöspores and resting spores were available to determine their size, structure and development and to show that the Brazilian fungus is morphologically identical to the one described by the author (**1941, 1942 a**) from Texas. However, no cross inoculations were made and it is not known whether or not the parasite from Brazil will infect other species of *Cladochytrium* and *Nowakowskiella*.

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and zoöspores of parasite; 12, unusually large septate terminal swelling from which zoöspores are emerging; 13, empty sporangium with extraneous residue; 14, 15, stages in resting spore development; 16, young incipient resting spore; 17–19, mature resting spores.

3. ROZELLA RHIZOPHLYCTII.

This chytrid was first described as a parasite of *Rhizophlyctis Petersenii* (Karling, **1942 a**) although Miss Ward (**1939**) had previously noted and figured a few resting spores of a parasite in *Rhizophlyctis rosea* which probably relate to the same species. What appears to be *R. Rhizophlyctii* was found by the author in sporangia of *Rhizophlyctis rosea* isolated on bits of onion skin from soil cultures collected in the Acre Territory, Brazil. Unfortunately, *Rhizophlyctis Petersenii* was not available in Brazil for cross inoculation experiments, and it is not certain that the South American fungus will infect this host. Nevertheless, it is so similar in structure and method of development to *Rozella Rhizophlyctii* that the writer regards the two species as identical.

Another *Rozella* parasite was found in an unidentified species of *Rhizophlyctis* collected in moist soil at Saõ Carlos, Matto Grosso. This host differs from *Rhizophlyctis rosea* by the presence of a large hyaline refractive globule in the zoöspores, and appears to be a new species. Its parasite, on the other hand, is identical to *Rozella Rhizophlyctii* in structure and method of development and will infect *Rhizophlyctis rosea*. However, no opportunity was available to test its pathogenicity to *Rhizophlyctis Petersenii*. The author, nonetheless, believes it is identical to *R. Rhizophlyctii*.

4. ROZELLA ENDOCHYTRII.

This species occurred as a parasite in the sporangia of *Endochytrium operculatum* which had been collected in dead grass leaves from a swamp at kilometer 53 on the Madeira-Mamoré railroad in the Matto Grosso. Like *R. Rhizophlyctii* this parasite does not cause hypertrophy or septation of the host sporangia. It differs primarily from the former species by the structure of its zoöspores and the fact that it is limited in host range to *E. operculatum* as far as is now known. Resting spores are unknown in this species, and none was found in the material from Brazil. The Brazilian parasite, nevertheless, agrees in all respects with the species found by the author (**1941**) in Texas, and the two fungi are, therefore, regarded as identical.

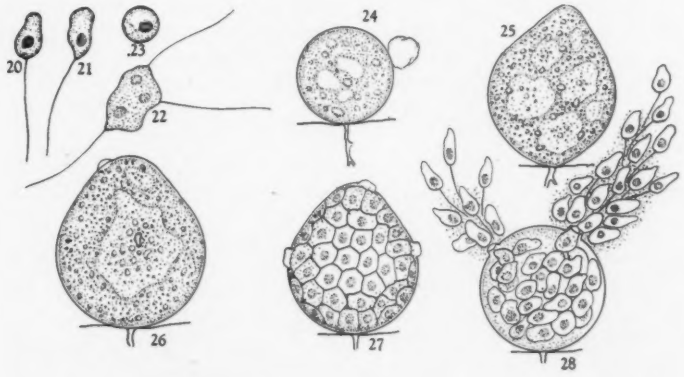
5. **Rozella Rhizophydii** sp. nov. (FIGS. 20–28).

Fungus parasiticus; sporangiis solitariis, longitudinem latitudinemque cellae matricolis in toto complentibus, sphaericis, 15–30 μ ovalibus, 10–12 × 13–20 μ, pyriformibus, 12–15 × 16–25 μ cum 1–3 papillis exeuntibus; pariete sporangii ex cella matricali fere non discernendo. Zoosporiis hyalinis, ovalibus aut obpyriformibus, 2–2.5 × 3–4 μ; flagello 12–14 μ longo. Sporiis perdurantibus non notis.

Sporangia solitary, filling host cell and conforming with the latter's size and shape, spherical, 15–30 μ, oval, 10–12 × 13–20 μ or pyriform, 12–15 × 16–25 μ with 1–3 low exit papillae; wall of sporangium indistinguishable from that of host. Zoöspores hyaline, oval or slightly pyriform, 2–2.5 × 3–4 μ; with a small globule near the posterior end; flagellum 12–14 μ long. Resting spores unknown.

Parasitic in *Rhizophydium globosum*, Manaus, Amazonas.

This species occurred in the sporangia of *Rhizophydium globosum* which in turn was parasitizing *Xanthidium subhastiferum*



FIGS. 20–28. *Rozella Rhizophydii*. 20, 21, motile zoöspores of *R. Rhizophydii*; 22, abnormal triflagellate zoöspore; 23, zoöspore after coming to rest; 24, infection of host; 25, 26, development of parasite inside of host sporangium; 27, host sporangium filled with zoöspores of parasite; 28, discharge of zoöspores.

in the swimming pool of the Bosque Club at Manaus. As far as is now known it appears to be an obligate parasite of this host, because all attempts to transfer it to *R. sphaerocarpum*, *R. pollinis*, *R. carpophilum*, *Endochytrium operculatum*, *Rhizophlyctis rosea*,

Cladochytrium replicatum, *C. crassum*, *Nowakowskiella elegans*, *N. ramosa*, *N. elongata*, and *N. granulata*, have failed.

The development and life cycle of this species, as far as it is known, are shown in figures 20 to 28. The structure and shape of the zoöspores (FIGS. 20, 21), method of infection (FIG. 24), development of the thallus within the host (FIGS. 25, 26), cleavage (FIG. 27), liberation and behavior of the zoöspores (FIG. 28) are so similar to those previously described for *R. Rhizophlyctii* and *R. Endochytrii* that very little can be added to the accounts already given. Like both of these species, *R. Rhizophydii* does not cause marked hypertrophy and distortion of the host cell nor thickening of its walls. Resting spores have not been found, so that comparisons on the basis of this character cannot be made at present. However, the zoöspores of *R. Rhizophydii* are slightly larger than those of either of the two former species. This difference coupled with the fact that the Brazilian parasite did not infect *Rhizophlyctis* and *Endochytrium*, leads the author to the conclusion that *R. Rhizophydii* is a distinct species.

SEPTIGENOUS POLYSPORANGIATE SPECIES

ROZELLA ACHLYAE.

So far this is the only polysporangiate species found in Brazil. What appears to be the same fungus was previously reported but not named by the author (**1942 b**, p. 204) as a parasite of *Achlya* in New York. Later in the same year Shanor (**1942**) gave a brief account of its development in *Achlya flagellata* and named it *R. Achlyae*. In Brazil it was found in the sporangia of the same host which had been isolated in water cultures from Rio Negro at Manaus, Amazonas. The size, shape and structure of the zoöspores, development of the sporangia and resting spores, and the effects produced in the host cell are similar to those described by Shanor, and there is no doubt in the author's mind that the two fungi are identical. This view is further supported by the fact that the Brazilian parasite also is limited in host range to *Achlya flagellata*.

SUMMARY

Five monosporangiate species of *Rozella*, *R. laevis* in *Pythium* sp., *R. Cladochytrii* in *Cladochytrim replicatum*, *R. Rhizophlyctii*

in *Rhizophlyctis rosea* and *Rhizophlyctis* sp., *R. Endochytrii* in *Endochytrium operculatum*, and *R. Rhizophydii* in *Rhizophydium globosum*, were found in Brazil. *Rozella Rhizophydii* has fairly large zoöspores and is limited in host range to *Rhizophydium globosum*. It is accordingly diagnosed as a new species, although its life cycle is not completely known. In addition to these monosporangiate parasites, one polysporangiate species, *R. Achlyae*, was found in the sporangia of *Achlya flagellata*.

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MYCOSPHAERELLA TULASNEI IN APPLE AND PEAR ORCHARDS

J. R. KIENHOLZ[1]

The relationship between *Cladosporium herbarum* Link and *Mycosphaerella Tulasnei* Jancz. was confirmed by Ruehle.[2] He produced the ascigerous form of the fungus by inoculating sterilized wheat leaves with the *Cladosporium* stage. The occurrence of perithecia on fallen apple and pear leaves appears not to have been reported.

In cold storage the fungus produces a slow rot of apple fruits and a more rapid rot of ripe pears. It may cause a blossom rot during heavy fogs in Russia according to Balakhonoff,[3] and occurs as a saprophyte almost everywhere. Perithecia of the fungus have commonly been observed in the spring on overwintered apple and pear leaves at Hood River, Oregon, since 1932. Partially developed perithecia were less frequently observed to develop in scurfy areas below buds on pear shoots which had grown rapidly during the past season. Typical *Cladosporium herbarum* cultures were obtained from ascospore isolations of this material and perithecia were again produced from cultures on sterilized leaves of wheat, apple, and pear in culture.

The following measurements and notes are based on fresh naturally occurring fruiting bodies from overwintered apple and pear leaves:

Perithecia: 100–160 μ wide by 150–250 μ deep; immersed in the leaf tissue from $\frac{1}{4}$ to $\frac{1}{3}$ of their depth; present on either leaf surface not in contact with the soil.

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[2] Ruehle, G. D. New apple-rot fungi from Washington. Phytopath. **21**: 1141–1152. 1931.

[3] Balakhonoff, P. I. (Note on the dying off of fruit tree blossoms on the Black Sea Littoral in connection with fogs.) Bull. North Caucasian Plant Prot. Station, Rostoff-on-Don. **1930**: 169–172. 1930. Abs. in Rev. Appl. Mycology **10**: 605. 1931.

Asci: 32–107 μ by 11–27 μ; mean 67.2 μ by 17.4 μ.

Ascospores: Typically 2-celled; 11–29 μ by 4–9 μ; mean 21.6 μ by 7.9 μ (top cell) to 6.4 μ (bottom cell); hyaline to pale olivaceous brown when older.

3-celled; mean 25.5 μ by 8.0 μ.
4-celled; mean 28.3 μ by 8.5 μ.

The ascospores usually become 3- to 4-celled during germination. Conidia produced in cultures or on fruit tissue were variable in shape, almost globose to narrowly ellipsoid; 1- to 3-celled; up to 30 μ in length; with a finely echinulate spore wall.

Specimens have been deposited in the mycological collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

ZOÖSPOROGENESIS IN THE RESISTANT SPORANGIA[1] OF ALLOMYCES ARBUSCULUS

WINSLOW R. HATCH[2]

(WITH 2 FIGURES)

INTRODUCTION

Our knowledge of the morphology of the resistant sporangia of *Allomyces arbusculus* can be quickly and thoroughly appraised by reviewing those facts which Emerson presented in his last paper (**1941**) but which, of necessity, he had to scatter through the many sections of his fine paper.

The resistant sporangia of *Allomyces arbusculus* are "more or less ovoid—with a flat, sometimes truncate, base. They are formed within the original hyphal membrane but are not fused to it and have a two-layered wall of their own, the outer of which is—thickened and brown."[3] The thick, outer wall possesses "fine, closely spaced pits" which are "distinct—averaging 1 μ apart." The "normally tawny to reddish brown" color of the resistant sporangia is "probably" caused by "a melanin-like substance." The resistant sporangia are "20–81 μ" in length; "16–60 μ" in width. They are "always produced in abundance" (on the asexual plant). "They are formed terminally—later becoming sympodially arranged; very rarely intercalary or in short chains." "Zoösporangia are normally formed first on young hyphae; heavy-walled (resistant) sporangia appear somewhat later depending on environmental conditions."[4]

In the dehiscence of resistant sporangia the thick outer wall cracks longitudinally. The "cracking of the outer wall is evidently a result of pressure from within, and the content, sur-

[1] While the term zoösporangia is undoubtedly the more correct word, the term "sporangia" is retained. For terminology see Emerson, 1941, p. 89.

[2] Part of this study was made at Dartmouth College, Hanover, New Hampshire.

[3] Emerson, 1941.

[4] Emerson, 1941.

rounded by a thin inner membrane, may continue to swell until it becomes more than twice its original diameter. One to four or occasionally more discharge papillae are formed, exactly similar to those on the zoösporangia and gametangia. Germination (= dehiscence), when once initiated, takes place within a short period, often less than sixty minutes after splits first appear in the outer wall. After most of the spores (= zoöspores) have emerged, the thick elastic outer wall of the sporangium frequently closes in again almost regaining the position which it originally occupied at the start of germination (= dehiscence). In so doing it may crumple the thin inner membrane and trap a few of the spores (= zoöspores) still remaining within." [4]

"These results indicate that reduction divisions and segregation of the parental characters take place after the formation of resistant sporangia on the sporophyte and before the development of mature gametophytes." [5]

While the writer (Hatch, **1938**) questioned this interpretation, suggesting that meiosis occurred in the germination of the zygote, a more careful analysis of his material, to be reported upon in another paper, has convinced him that there is really no evidence for meiosis at zygote germination.

The process of zoösporogenesis in resistant sporangia on asexual (sporophytic) plants is a critical process, because meiosis occurs during zoösporogenesis and because the sexual or asexual condition of the plants derived from R. S. zoöspores [6] is apparently determined during zoösporogenesis. As has been suggested above, we know a good deal about the morphology of resistant sporangia. We know something of the process of dehiscence, a stage in zoösporogenesis, but we need to know more about the morphology, cytology, and physiology of zoösporogenesis in resistant sporangia. This paper constitutes the first of a series dealing with this process.

MATERIAL AND METHOD

The source of the material used in this study was *Allomyces arbusculus*, North Carolina isolate No. 2.[7] The resistant spor-

[5] Emerson, 1941.

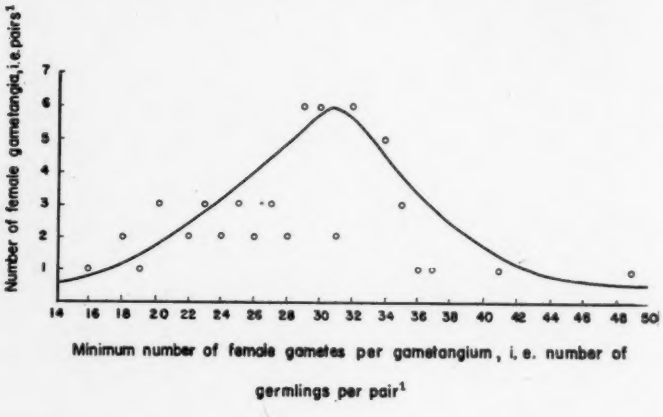
[6] Zoöspores from resistant sporangia, Emerson's abbreviation.

[7] Emerson, 1941. p. 82.

angia studied were grown in water cultures, baited with hemp seed and on maltose-peptone agar plates. The resistant sporangia were dried for different periods of time, introduced into glass-distilled water and then brought under observation under supported cover slips.

RESISTANT SPORANGIA INCAPABLE OF ZOÖSPOROGENESIS

When dried, resistant sporangia from either water or agar cultures are placed in distilled water or in a maltose-peptone solution and then are brought under observation, it is relatively


Number of female gametangia, i.e. pairs[1]
7
6
5
4
3
2
1
14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
Minimum number of female gametes per gametangium, i.e. number of germlings per pair[1]

[1] Couplets of 1 male and 1 female gametangium

FIG. 1.

easy to determine which of the resistant sporangia are incapable of zoösporogenesis because these resistant sporangia become filled with several relatively large spheres which are sometimes of nearly uniform size (FIG. 2, *a*), at other times are of noticeably different size (FIG. 2, *b*). These spheres are apparently formed by the enlargement and coalescence of "lipoid granules." [8] The "lipoid granule" is a normal constituent of the cytoplasm of *Allomyces arbusculus*. In living, healthy cytoplasm the "granules" are small. When the cytoplasm in a hypha, gametangium, or spor-

[8] This is Guilliermond's term (1920), (1922), (1922). Actually they are more like "droplets" in shape and consistency.

angium breaks down or when a gamete or zoöspore dies, the first morphological manifestation of death is a marked increase in the size of the "granules" (Hatch, **1935**). From these observations it seems very likely that resistant sporangia in which these enlarged spheres appear could not be brought to dehiscence under any condition, for they are, in all probability, no longer alive.

THE PROCESS OF ZOÖSPOROGENESIS IN RESISTANT SPORANGIA

Resistant sporangia capable of zoösporogenesis undergo a series of metamorphoses that parallel those experienced in gametogenesis (Hatch, **1933**, **1935**). When resistant sporangia are ready to undergo zoösporogenesis, a change in their appearance—the initial change—may be expected in approximately 10 minutes after they have been introduced into distilled water or maltose-peptone solutions. At this time the resistant sporangia take on a granular appearance. After approximately 2 hours this stage gives way to a phase in which the peripheral cytoplasm of the resistant sporangia becomes studded with "circles." In another 30± minutes these "circles" disappear and the resistant sporangia again become granular in appearance. This stage lasts only for a few minutes before the outlines of the R. S. zoöspores [9] become apparent. Dehiscence, meaning the swelling of the R. S. zoöspores and the cracking of the resistant sporangium wall, takes approximately 60 minutes. It has become a convention to speak of these stages as the *granular*, *zoöspore origin*, *disappearance*, *cleavage*, and *dehiscence* stages respectively.

An aspect of zoösporogenesis in resistant sporangia to which attention should be called is the fact that the process itself is not ordinarily initiated in the culture where the resistant sporangia are formed, or if initiated, it certainly does not proceed beyond the *pre-granular* stage. The word "ordinarily," last sentence, is used advisedly, because under certain conditions resistant sporangia do undergo zoösporogenesis in water cultures. Gametogenesis and zoösporogenesis in zoösporangia, on the other hand, are characteristically carried to completion in water cultures. In agar cultures they are halted in the *gamete* or *zoöspore origin* stage. In water cultures where conditions are such as to discourage or

[9] Zoöspores from resistant sporangia, Emerson's abbreviation.

prohibit dehiscence these processes are likewise brought to at least a temporary stop. In these instances where gametogenesis and zoösporogenesis in zoösporangia are halted, it is well to observe that the processes in question always reach the *origin* stage in their development—always complete their nuclear divisions. A second peculiarity of zoösporogenesis in resistant sporangia that will stand emphasis is the fact that it does not occur ordinarily until the resistant sporangia have been dried.

The fact that zoösporogenesis in resistant sporangia does not ordinarily occur until resistant sporangia have been dried and/or placed in fresh water or nutrient solutions makes it possible for the experimenter to isolate this process in point of time and place and so control the conditions under which it is initiated and under which it proceeds. Since meiosis regularly occurs during this process, we have in the resistant sporangia of *Allomyces* structures in which the phenomenon of meiosis can be most advantageously studied.

GRANULAR STAGE (FIG. 2: c_1, c_2, c_3, c_4)

While resistant sporangia in the *granular* stage appear superficially to be granular throughout, closer observation shows that the cytoplasm is very vacuolate, that the granules ("lipoid granules") are dispersed over the faces of intervacuolar films. Actually, the cytoplasm of resistant sporangia is so vacuolate that the nuclei or the nuclear spindles, as the case may be, are often elongated, twisted or otherwise distorted.

The *granular* phase is the longest and the most critical phase of zoösporogenesis. During this phase the "lipoid granules" decrease in size. Initially there may be no more than 5–12 nuclei in resistant sporangia, the number found in the hyphal tips when the resistant sporangia are formed. Ultimately the number may become as great as 88± to 136± or even perhaps 164±. The nuclei are at first large, at least as large as the nuclei found in the hyphae. After the *granular* stage has run its course, the nuclei become very small. It is obvious that these nuclei have undergone many mitoses and, probably, meiosis as well. In the granular stage the vacuoles also experience a gradual change, consolidating toward the center of the resistant sporangia, forcing

the nuclei to the periphery. It is this change which brings about the altered appearance of the sporangia which is conventionally described as the ***zoöspore origin*** stage.

ZOÖSPORE ORIGIN STAGE (FIG. 2: *d*, *e*)

The so-called *origins* are the nuclei. Since the nuclei at this stage are forced against the wall and since they attract to their respective surfaces the very numerous "lipoid granules," they look like circles outlined by dots. Since the full nuclear complements of resistant sporangia are achieved by this time, the number of nuclei produced is the same as the number of R. S. zoöspores that will ultimately be formed. In this sense, but in this sense only, can these nuclei be considered zoöspore origins. The number of nuclei or origins in resistant sporangia is large.

DISAPPEARANCE AND CLEAVAGE STAGES (FIG. 2, *f*)

After approximately 30 minutes in the ***zoöspore origin*** stage, the cytoplasm becomes organized about the nuclei, cleavage furrows come in and the R. S. zoöspores are cut out. The number of R. S. zoöspores formed is, of course, the same as the number of nuclei or *origins* formed at the onset of the ***zoöspore origin*** stage. The swelling of resistant sporangia, so characteristic of the process of zoösporogenesis in resistant sporangia, does not begin until the R. S. zoöspores have been completely delimited.

DEHISCENCE (FIG. 2, *g*)

The process of dehiscence has been described by both Emerson (**1941**) and Sörgel (**1937**), and while Emerson describes the process under the name "germination," his interpretation and the writer's are the same. Dehiscence involves the swelling that cracks the thick middle wall as well as the cracking itself. According to Emerson, the process of dehiscence (*i.e.* the whole process) takes approximately 60 minutes from the cracking of the thick middle wall to the discharge of the first R. S. zoöspore.

THE R. S. ZOÖSPORE (FIG. 2, *h*)

The structure of an R. S. zoöspore is not unlike that of a gamete, in that it normally has a single cilium, a single nucleus, a nuclear

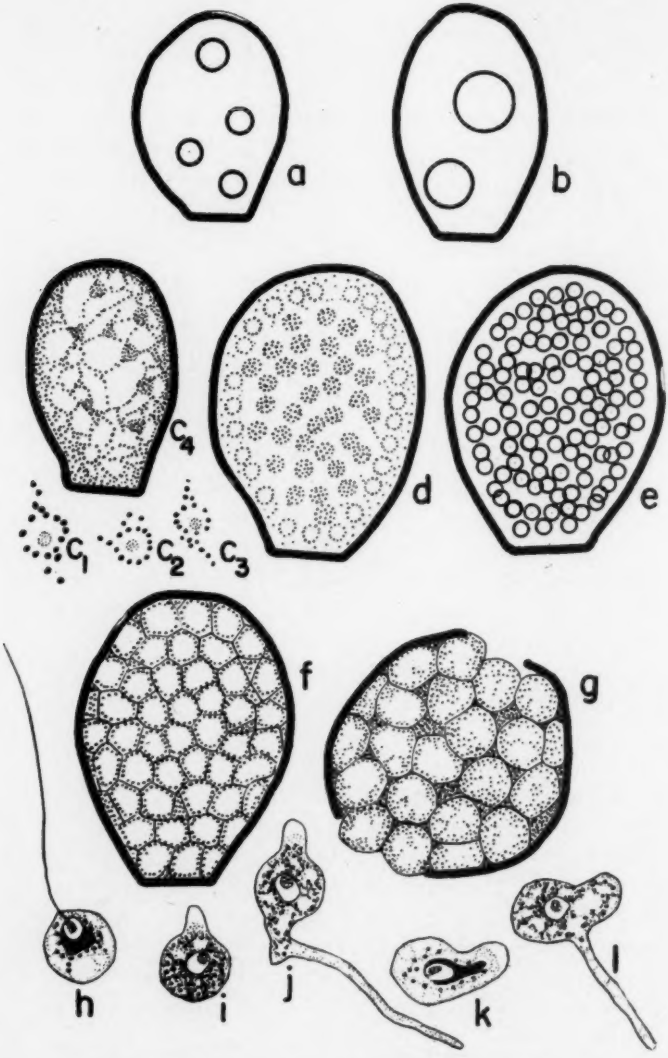


FIG. 2. Zoösporogenesis in resistant sporangia. See page 663, explanation of figures.

cap, and dispersed "lipoid granules." The cilium is inserted on the R. S. zoöspore membrane and has a rhizoplast extension which ultimately connects with the nucleolus in precisely the same way as it does in the gamete. The nuclear cap seems small but whether it is proportionately smaller than the same structure in the gamete is not easily determined. The R. S. zoöspore's quota of "lipoid granules" is apparently not as great as that of the gamete.

The swarming habits of R. S. zoöspores vary. Some swim for but a minute or two; others for 15 to 20 minutes; while still others alternately swim and crawl for better than two hours. These differences in swarming habit are found even among the products of a single resistant sporangium.

The history of the R. S. zoöspores that swim for a few minutes is shown in figure 2; *k*, *l*. These R. S. zoöspores, always a relatively small number, become amoeboid soon after their discharge from the resistant sporangia. They often persist in their amoeboid movements up to the very moment of their germination. In fact, encystment and germination appear to be almost simultaneous because the R. S. zoöspores do not always round up before pushing out their germ tubes.

Those R. S. zoöspores that swarm for 15 to 20 minutes characteristically round up in the process of encystment, but even among these R. S. zoöspores encystment precedes the extension of the germ tube by only a matter of minutes. In R. S. zoöspores of this type (FIG. 2: *i*, *j*) the term tubes are often clearly visible 12 minutes after encystment.

Those R. S. zoöspores that swim for more than two hours do not swim about for the whole period but lapse into several prolonged periods of amoeboid activity. These R. S. zoöspores have never been observed to germinate.

When R. S. zoöspores encyst, the movement of the cilium stops instantly—the cilium is apparently cast. The process of R. S. zoöspore germination is not unlike that of the zygote, in that the nuclear cap becomes disassociated prior to or just after the development of the germ tube.

The time table for zoösporogenesis is apparently very variable. In some instances and under certain conditions dehiscence is

accomplished in 2½ hours after the resistant sporangia are introduced into distilled water or maltose-peptone solutions. In other instances and under other conditions dehiscence has not been observed before 4, 8, 12 and even 20 hours.

NUMBER OF ZOÖSPORES IN RESISTANT SPORANGIA

The number of R. S. zoöspores that will be produced in a given resistant sporangium is most easily determined by counting the nuclei in the *zoöspore origin* stage (FIG. 2: *d*, *e*). After the R. S. zoöspores have been delimited it is more difficult, if not impossible, to count them, and at any time previous to the *zoöspore origin* stage any count of nuclei would be without meaning in determining R. S. zoöspore number because the nuclei would not as yet have completed their nuclear divisions. In the *zoöspore origin* stage the nuclei can be counted with accuracy because of their peripheral position and because they are outlined by lipoid granules.

The number of nuclei formed in resistant sporangia is relatively large. Kniep (**1930**) observed that resistant sporangia ultimately produced a great many nuclei, but his observation does not prepare one for counts of 88± to 136±, let alone counts of 164±.

Counts made upon female gametangia in the same stage of division, namely, the *gamete origin* stage, are known to possess from 12 to 36 nuclei (Hatch, **1935**). While no higher counts have been made directly on female gametangia, we have reason to believe that some large female gametangia may possess as many as 41 and possibly as many as 49 gametes.

A comparison of resistant sporangia and female gametangia as regards their nuclear complements can not be made without taking into account the relative volumes of the two structures. To this end a single resistant sporangium in the *zoöspore origin* stage and a single female gametangium in the *gamete origin* stage were compared. The volumes and the nuclear counts of these two structures are recorded in table I.

The volumes given in the table were arrived at by using the formula $4/3\ xy^2\pi$, in which "x" equals the long radius and "y," the short. In this, the formula for the volume of a prolate

spheroid, a portion at the base of both the resistant sporangium and female gametangium is not calculated. The volumes then do not represent the actual volumes of the two structures, but something less. It is for this reason that the plus sign (+) appears after the figures. While the volumes determined through the use of this formula do not represent the actual volumes of the structures in question, they do indicate *relative volumes*. Since the relative volumes are all that are needed in this analysis, the formula seems adequate, if not entirely satisfactory. Calculating the volumes of these structures as though they were spheres would, it would seem, be even less satisfactory. Turning now to table I we find that the volume of the resistant sporangium was 11,750 + cu. μ, its nuclear number 104±. The volume of the female gametangium was 21,750 + cu. μ, its nuclear number 36.

While the volume of the resistant sporangium described in table I is considerably smaller than the volume of the female gametangium with which it is compared, it must not be concluded that resistant sporangia are regularly smaller than female gametangia. As a matter of fact, when large enough samples are studied, resistant sporangia and female gametangia appear to have approximately the same volume. The volume of a resistant sporangium of mean size, using Emerson's measurement but correcting for the thickness of the middle wall, is 22,430 cu. μ. No statistical study has been made of female gametangia but if we consider the figures on the minimum number of female gametes per gametangium described in the paragraph below and shown in figure 1, we find that a gametangium possessing 36 gametes is only 25± per cent above the mean. Since the figures referred to above deal with the minimum number of female gametes per gametangium, it is probable that a female gametangium with 36 gametes is even nearer the mean. Lacking a complete statistical study of female gametangia, it is probably reasonable to conclude that the volume of the female gametangium considered in table I, namely 21,750 cu. μ, is near the mean for female gametes and can be compared with the mean volume for resistant sporangia, namely 22,430 cu. μ.

The number of nuclei counted in the resistant sporangium described in table I was 104±. The number of nuclei in the

TABLE I

COMPARATIVE FIGURES ON A RESISTANT SPORANGIUM AND A FEMALE GAMETANGIUM

	Volume	*Origin* stage	Cleavage stage
		Number of *origins* or nuclei	Volume of zoöspores and gametes
Resistant sporangium.....	11,750+cu. μ*	104±	113+cu. μ‡ (zoöspores)
Female gametangium.....	21,750+cu. μ†	36	604+cu. μ§ (gametes)

* Measurements from inner face of the thick wall = 36.4 × 24.8 μ.

† Measurements = 34.9 × 34.4 μ.

‡ Volumes estimated by dividing vol. resistant sporangium by no. of origins.

§ Volumes estimated by dividing vol. female gametangium by no. of origins.

resistant sporangia found on the plant with this sporangium varied between 88± and 136±. In addition to these studies counts were made upon the products of single resistant sporangia. The number of plants formed from the R. S. zoöspores produced by individual resistant sporangia varied between 109+ and 164±.

From the evidence of nuclear counts made in the *zoöspore origin* stage and from single resistant sporangia isolations, we can conclude that *104*± is less than the mean for the number of nuclei in resistant sporangia.

The number of nuclei counted in the female gametangium described in table I was 36. In earlier counts by the writer (Hatch, **1935**), on a small sample, the number varied between 12 and 36. In a series of experiments undertaken in connection with quite another problem, 63 pairs of co-joined male and female gametangia were isolated. From 55 of these pairs numerous asexual germlings developed. Since the female gametangia involved in these isolations may have possessed more gametes than is to be deduced from the number of germlings produced by each isolated pair, the number quoted in each case represents the minimum number of gametes. The mean number of female gametes per gametangium, *i.e.*, the number of germlings per pair, and the statistical distribution of these numbers is shown in

TABLE II

COMPARATIVE VOLUMES FOR R. S. ZOÖSPORES AND FEMALE GAMETES

	At cleavage		At swarming
R. S. Zoöspores	110+–134+ cu. μ*	↔	223–606 cu. μ‡
Female gametes	288–675 cu. μ†		(288–675 cu. μ)§

* Vols. estimated by dividing vols. resistant sporangia by no. of their origins.

† Vols. essentially same as for swarming gametes. See §.

‡ Vols. computed from Emerson's measurements. Mean diam. = 7.5–105 μ.

§ Vols. computed from Emerson's measurements. Mean diam. = 8.2–10.8 μ.

figure 1. The minimum number of female gametes per gametangium, *i.e.*, the mean number of germlings per pair, was 28.7—the minimum 16, the maximum 49. From this we can conclude that 36 is above the mean number for female gametangia.

In sum, when all nuclear division has ceased in resistant sporangia, the number of nuclei or *zoöspore origins* is approximately four times as great as the number of nuclei or *gamete origins* found in female gametangia, structures of approximately the same volume. The actual counts were 88± to 136± for resistant sporangia, 12 to 36 for female gametangia.

VOLUME OF ZOÖSPORES IN RESISTANT SPORANGIA

The relative volumes of R. S. zoöspores and female gametes are shown in table I, last column, and in table II.

In table I, last column, the volume of the zoöspores in a single resistant sporangium are compared with the volume of the gametes in a single female gametangium. The volume of the R. S. zoöspores was 113+ cu. μ,* that of the female gametes 604+ cu. μ.*

In table II the volumes of R. S. zoöspores and female gametes are compared. From a study of the table it is apparent that the volume (110+*–134+* cu. μ) of R. S. zoöspores at cleavage is ap-

* These volumes are greater than stated because they have been calculated from the volumes of resistant sporangia and female gametangia which were greater than could be computed.

proximately one-fourth as great as the volume (288–675 cu. μ) of female gametes at cleavage; is approximately one-fourth as great as the volume (223–606 cu. μ) of swarming R. S. zoöspores.

The relative volumes of the nuclei of R. S. zoöspores and female gametes are shown in table III. From a study of the table it is apparent that the nuclei of R. S. zoöspores and female gametes compare as do the zoöspores and gametes themselves. The volume (2.7–4.7 cu. μ) of the nuclei of the R. S. zoöspores at cleavage is approximately one-fourth as great as the volume (9.0–25.3 cu. μ) of the nuclei of female gametes at cleavage; is approximately one-fourth as great as the volume (10.8–17.3 cu. μ) of the nuclei of swarming R. S. zoöspores.

TABLE III

COMPARATIVE VOLUMES FOR THE NUCLEI OF R. S. ZOÖSPORES AND FEMALE GAMETES

	At cleavage		At swarming
R. S. zoöspore, nuclei	2.7–4.7 cu. μ*	$\leftrightarrow$	10.8–17.3 cu. μ‡
	$\updownarrow$		
Female gamete, nuclei	9.0–25.3 cu. μ†		(9.0–25.3 cu. μ)§

* Volumes computed from mean diameters of 1.7–2.0 μ.
† Volumes essentially same as for swarming gametes. See §.
‡ Volumes computed from mean diameters of 2.7–3.2 μ.
§ Volumes computed from mean diameters of 2.9–3.6 μ.

SUMMARY

(1) Zoösporogenesis in resistant sporangia is not normally initiated until the resistant sporangia have been dried and/or placed in fresh water or nutrient solution.

(2) The *number* of R. S. zoöspores per resistant sporangium is approximately 4 times as great as the number of female gametes produced in the female gametangium, a structure of approximately the same volume.

(3) The *volume* of R. S. zoöspores at cleavage is approximately one-fourth as great as the volume of female gametes at cleavage.

(4) The *volume* of R. S. zoöspores at cleavage is approximately one-fourth as great as their volume when swarming.

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EXPLANATION OF FIGURES

FIG. 1. Minimum number of female gametes per gametangium.

FIG. 2. Zoösporogenesis. (*a–g.*) Resistant sporangia. The thin outer and inner walls are not shown. The thick middle wall is drawn in median optical section; its sculpturing is not shown. *a* and *b*. Resistant sporangia incapable of zoösporogenesis. Note characteristic spheres probably enlarged "lipoid granules." *c*. *Granular* stage: c_1, c_2, c_3, c_4, stages showing the progressive decrease in the size of the nuclei and "lipoid granules." (Lipoid granules outline nuclei.) In the resistant sporangium two nuclei are shown in outline, 7 as local concentrations of "lipoid granules." *d*. *Zoöspore origin* stage: Nuclei (*origins*) at a median focus are outlined by "lipoid granules." Those in the face toward the observer are studded with "lipoid granules." Total number nuclei (*origins*) = 88; 28 nuclei on the face away from the observer are not drawn. *e*. Full quota nuclei (*origins*) for a resistant sporangium. Total number = 104. *f*. Cleavage stage. *g*. Dehiscence. *a–g* = ×1055. *h*. R. S. zoöspore, motile. Note vacuolate cytoplasm, small number of "lipoid granules," nuclear cap, nucleus, cilium with rhizoplast. *i* and *j*. Germinated, R. S. zoöspores. *k*. Amoeboid R. S. zoöspore. *l*. Germinated, R. S. zoöspore. A few R. S. zoöspores germinate without rounding up. *h–l* = ×1521.

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TORREY, G(EORGE) SAFFORD, Professor of Botany, University of Connecticut; *R. F. D. 2, Storrs, Conn.* (Intestinal fungi of Arthropods.)

TUCKER, DR. C(LARENCE) M(ITCHELL), Chairman, Department of Botany, University of Missouri, *100 Lefevre Hall, Columbia, Mo.* (Phycomycetes; plant pathology.)

TULLIS, DR. EDGAR C., Pathologist, U. S. Department of Agriculture, Cereal Crops and Diseases, *P. O. Box 2967, Beaumont, Texas.* (Rice disease.)

UMANZIO, CARL B(EEMAN), Assistant Professor of Bacteriology, *77 Ramshead Road, Medford, Mass.* (Medical mycology.)

VANTERPOOL, T(HOMAS) C(LIFFORD), Professor of Plant Pathology, *Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.* (Root rots of cereals, particularly those caused by Pythium; flax diseases.)

VERMILLION, M(ONROE) T(HOMAS), Instructor in Botany, Ohio University; *Earich Road, R. D. 3, Athens, Ohio.*

WAKSMAN, DR. SELMAN A., Professor and Head, Department of Soil Microbiology, *New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, N. J.* (Soil mycology; Actinomycetes; physiology of fungi; production of antibiotic substances by microörganisms.)

WALKER, DR. A(NSON) R(OBERTSON), Professor of Plant Pathology, *Department of Botany, University of Western Ontario, London, Ontario, Canada.* (Comparative morphology.)

WALKER, DR. LEVA B(ELLE), Associate Professor of Botany, *University of Nebraska, Lincoln 8, Nebr.* (Morphology.)

WALTERS, MAURICE B., *14556 Superior Road, Cleveland Heights, Ohio.*

WATERMAN, DR. ALMA M(AY), Assistant Pathologist, U. S. Department of Agriculture, *360 Prospect St., New Haven, Conn.* (Diseases of shade and ornamental trees.)

WATERSTON, J. M., Plant Pathologist, *Department of Agriculture, Agricultural Station, Paget East, Bermuda.* (Mycology; plant pathology.)

WEBER, DR. GEORGE F(REDERICK), Professor of Plant Pathology, *University of Florida, Gainesville, Fla.* (Parasitic fungi; taxonomy.)

WEHMEYER, DR. LEWIS E(DGAR), Associate Professor, Department of Botany, University of Michigan; *381 Orchard Hill Dr., Ann Arbor, Mich.* (Pyrenomycetes.)

WEIDMAN, DR. FRED(ERICK) D(EFOREST), *Medical Laboratories, University of Pennsylvania, 36th St. and Hamilton Walk, Philadelphia, Pa.* (Medical mycology and dermatology.)

WELCH, DR. DONALD STUART, Professor in Plant Pathology, *New York State College of Agriculture, Cornell University, Ithaca, N. Y.* (Taxonomy of Pyrenomycetes and Polyporaceae; forest pathology.)

WELLMAN, DR. F(REDERICK) L(OVEJOY), *10108 Pierce Drive, Silver Spring, Md.* (Physiology; morphology; variability; parasitism.)

WERNHAM, DR. CLIF(FORD) C(HARLES), *Department of Botany, Pennsylvania State College, State College, Pa.* (Genetics and physiology.)

WEST, ERDMAN, Mycologist in charge of Herbarium, *Florida Agricultural Experiment Station, Gainesville, Fla.* (Taxonomy of Florida fungi; timber decaying fungi; Myxomycetes; rusts; Sclerotium Rolfsii; Florida plants.)

**WESTON, DR. WILLIAM H., JR., Professor of Cryptogamic Botany, Harvard University, *Biological Laboratories, 16 Divinity Ave., Cambridge 38, Mass.* (Phycomycetes; scientific interest and economic importance of fungi.)

WHELDEN, DR. R(OY) M(AXFIELD), Harvard University, *Biological Laboratories, Divinity Ave., Cambridge, Mass.* (Cytology; radiation effects.)

WHETZEL, PROF. H(ERBERT) H(ICE), Professor of Plant Pathology, *Cornell University, Plant Science Building, Ithaca, N. Y.* (Taxonomy and morphology of Sclerotiniaceae.)

WHIFFEN, ALMA J., *Research Laboratories, Upjohn Company, Kalamazoo, Mich.* (Phycomycetes; penicillin.)

WHITE, DR. W. LAWRENCE, Research Associate in Chemical Warfare, Chemical Warfare Service Development Laboratory, Massachusetts Institute of Technology; *Farlow Herbarium, Harvard University, Cambridge, Mass.* (Mold-proofing military equipment for use in tropics.)

WOLF, DR. FREDERICK A., Professor of Botany, *Duke University, Durham, N. C.* (Morphology; taxonomy.)

WOLF, DR. FRED(ERICK) T(AYLOR), Assistant Professor of Botany, *Department of Biology, Vanderbilt University, Nashville, Tenn.* On leave of absence. (Phycomycetes.)

WOLFF, MISS EMILY T(OWER), Research Botanist, *American Cyanamid Co., Stamford, Conn.* (Commercial problems involving plant materials, referred to microscopy laboratory.)

WOOLLIAMS, G(EORGE) E(WART), Agricultural Scientist, Dominion of Canada, Department of Agriculture, *Dominion Laboratory of Plant Pathology, Summerland, British Columbia, Canada.* (Taxonomy; pathology.)

WRIGHT, C(HARLES) M(ILTON), Research Assistant, *Department of Plant Pathology, Cornell University, Ithaca, N. Y.*

YARWOOD, DR. CECIL E(DMUND), Assistant Professor of Plant Pathology, *Division of Plant Pathology, University of California, Berkeley, Calif.* (Powdery mildews; downy mildews.)

YAW, MISS CATHERINE EMILY, Research Mycologist, Parke-Davis and Co.; *427 E. Grand Blvd., Detroit, Mich.*

YORK, HARLAN H(ARVEY), Professor of Botany, *Department of Botany, University of Pennsylvania, Philadelphia, Pa.* (Forest pathology.)

ZELLER, DR. S(ANFORD) M(YRON), Plant Pathologist, *Oregon Agricultural Experiment Station, Oregon State College, Corvallis, Ore.* (Parasitic fungi and Gasteromycetes.)

ZUNDEL, DR. GEORGE L(ORENZO INGRAM), Assistant Professor of Plant Pathology, Extension Division, *203 Botany Building, Pennsylvania State College, State College, Pa.* (Ustilaginales.)

MEMBERS DECEASED
1943–1944

BULLER, ARTHUR HENRY REGINALD
BURNHAM, STEWARD HENRY
GOLDSMITH, GLENN WAREN
HENRICI, ARTHUR TRAUTWEIN
HORNE, WILLIAM TITUS

CONSTITUTION AND BY-LAWS

CONSTITUTION

Art. 1. Name. The Society shall be known as the Mycological Society of America.

Art. 2. Membership.

(1) The Society shall consist of members and may include life members, patrons, honorary members, and corresponding members.

(2) Charter membership in the Society shall consist of the persons who, after the invitation of the Secretary, joined before or during the formal organization of the Society at the Atlantic City meetings in 1932.

Art. 3. Dues. The dues for regular members shall be five dollars a year. Any member may become a life member by paying one hundred dollars in one payment, or a patron by paying one thousand dollars, and upon election shall have all the privileges of members. Such funds obtained from life members and patrons shall constitute an endowment fund to be used as may be decided by the Council for the support of mycological publications or projects.

Annual dues of five dollars shall include subscription to the official organ of the Society, and shall be payable in advance on or before December 20. Bills for dues shall be sent to the members in October and it will be necessary to discontinue sending the journal to those whose dues have not been paid by December 20.

Art. 4. Membership and Election of Members.

(1) All persons interested in the study of the fungi shall be eligible to membership.

(2) Members may be elected at any regular meeting of the Society or in the interim between meetings may be elected by the Council. Application for membership must be endorsed by at least one member of the Society.

Art. 5. Officers. The officers of the Society shall consist of a President, Vice-President, and Secretary-Treasurer, whose duties shall be those usually performed by such officers. The President and Vice-President shall serve for one year and the Secretary-Treasurer for three years (or until their successors are elected). Any vacancies occurring in the interim between elections shall be filled by the Council.

The Council shall consist of the President, Vice-President, Secretary-Treasurer, and four Councilors. The Councilors shall be elected, two each year, to serve a term of two years. Two of the councilors shall be from east of the Mississippi (Minnesota is counted as west of the Mississippi) and two west. An individual may not hold two or more positions on the Council at one time.

The Council shall name a Historian to serve for an indeterminate period of years. It shall be the duty of the Historian to accumulate and preserve facts, papers, photographs, and other materials pertinent to a permanent historical record of the Society. The Historian shall not become a member of the Council by virtue of his office as Historian.

Art. 6. Editors and Committees. The editors of the official journal of the Society shall be elected by the Council. The President shall appoint all temporary committees that are to serve during his administration and shall fill all vacancies on standing committees that may occur during his term of office.

Art. 7. Election of Officers. The Secretary-Treasurer shall send to each member of the Society in October a ballot for the nomination of officers. If any nominations are lacking, the Council shall have power to make them. The three candidates for each office receiving the highest number of nominating votes shall be placed upon a final ballot to be sent to each member December 1. Should the nominating votes received by a candidate place him among the highest three for more than one office, his name shall appear on the final ballot for only the highest office. The offices rank in the order given in article 5. Votes shall be mailed to the Secretary-Treasurer and counted by the Council. A plurality vote shall elect.

Art. 8. Meetings. An annual meeting shall be held at such time and place each year as the Council may select (usually in connection with the A.A.A.S. meetings). An additional meeting for informal discussion and the carrying out of collecting forays shall be held in the summer or autumn at a time to be selected by the Council. Additional meetings, including special or local meetings for the presentation of papers or the carrying out of forays, may be arranged by the Council at its discretion.

Art. 9. Divisions. Branch organizations or units within the Society known as Divisions, may be established on a geographical basis provided formal application, setting forth the reasons for the establishment of the Division, is made to the parent Society and approved by it.

Art. 10. Journal. The Society shall adopt or establish a journal which shall serve the Society as its official organ primarily for the publication of mycological papers by its members, for the publication of abstracts of the papers delivered at the annual or other meetings, and for the publication of the report of the Auditing Committee or of other reports, announcements, and business of the Society.

Art. 11. Amendments. These articles may be amended by a majority vote of the members voting at any regular meeting of the Society, provided that suggested amendments have been brought to the attention of the Council of the Society in time to be sent to all of the members at least one month previous to the meeting.

BY-LAWS

1. Programs. Programs for annual or other meetings shall be arranged by the Council.

2. Papers. Members wishing to present papers at the annual meeting shall submit to the Secretary-Treasurer the substance and conclusions of the papers in a clear and concise abstract of not more than 200 words. These

shall be due on or before November 15, and the Secretary-Treasurer shall be authorized to refuse any received after that date. These abstracts will be edited by the editorial committee of the official journal of the Society for subsequent publication in that organ. Members are urged not to submit titles or abstracts unless they expect to attend the meetings. Except by invitation no member shall offer more than two papers at any one meeting, papers of joint authorship being attributed to the author reading the paper.

3. Associates. Students and others not yet members of the Society may attend meetings and forays in the status of Associates, provided they are recommended to the Council by a member of the Society and pay a fee of one dollar. Such Associates, as they are not members, shall not have the privilege of voting and shall not receive the official journal of the Society, but shall enjoy the other privileges of the meetings and forays including the right to present one paper on the program.

4. Auditing. At each annual meeting the active President shall appoint an auditing committee to audit the accounts of the Society and of its official publication. An audited statement shall be published in the official organ of the Society.

5. Use of the Society's name. Unauthorized use of the name of the Mycological Society of America for advertising or other business ventures is prohibited. The circulation of any unauthorized literature shall be taken as prima facie evidence of the violation of the intent and purpose expressed in this by-law, and the member, after being properly notified, may be expelled from the Society by a majority vote of either the Society at its meetings, or by a majority vote of the Council.

6. These rules may be amended by a majority vote of the members voting at any regular meeting of the Society, provided that suggested amendments have been brought to the attention of the Council of the Society in time to be sent to all the members at least one month previous to the meeting.

CONTRACT WITH THE NEW YORK BOTANICAL GARDEN

The Mycological Society of America hereby adopts Mycologia as its official organ on the following terms:

1. Mycologia will continue to be published by the New York Botanical Garden, the editorial policies to be determined by an Editorial Board, consisting of a Managing Editor appointed by the New York Botanical Garden, and five Editors elected by the Mycological Society of America. The term of office of the five elected editors will be five years, except that at the start they will be designated to serve one to five years respectively. One editor will be elected annually, thereafter, to fill the place of each retiring editor.

The six members of the Editorial Board will elect an Editor-in-Chief from among their number. He will be eligible for repeated reëlection. Final decision of all questions on editorial policy will be made by him, except that the Managing Editor will have full authority in all matters pertaining to the finances of the journal.

2. All personal subscribers now receiving Mycologia may become members of the Mycological Society of America if they so desire. Institutional subscribers to Mycologia are not to be regarded as members of the Society.

3. All members of the Mycological Society of America in good standing will receive Mycologia. In return the Society will transmit to the New York Botanical Garden, through the Managing Editor, four dollars per year for each such member.

4. The New York Botanical Garden agrees to spend on the publication and distribution of Mycologia all funds received from subscriptions, as well as all funds transmitted by the Mycological Society of America. The Garden further agrees to use for these purposes all sums received from the sale of those volumes of the journal which shall be published after this contract is put in force. Earlier volumes remain the property of the New York Botanical Garden. It is understood that the journal will be used by the Garden for exchange purposes as formerly. Should the contract be terminated, it is agreed by the Mycological Society of America that all excess stock of Mycologia then on hand will be regarded as the property of the New York Botanical Garden.

5. The New York Botanical Garden reserves the fourth cover page to be used without charge for the advertisement of its publications, including Mycologia. The other three cover pages will be used by the Mycological Society of America as it may see fit. All sums collected from paid advertising will be expended on the journal.

6. This contract may be altered at any time by mutual agreement of the New York Botanical Garden and the Mycological Society of America. It may be terminated at the end of any calendar year on six months written notice should it prove unsatisfactory to either party concerned.

7. The contract goes into effect at the beginning of the calendar year 1933.

PAST AND PRESENT OFFICERS

	PRESIDENT		VICE-PRESIDENT
1932	Wm. H. Weston, Jr.	1933	G. W. Martin
1933	C. L. Shear	1934	B. O. Dodge
1934	H. S. Jackson	1935	John Dearness
1935	B. O. Dodge	1936	A. H. R. Buller
1936	H. M. Fitzpatrick	1937	L. O. Overholts
1937	John Dearness	1938	E. B. Mains
1938	L. O. Overholts	1939	D. H. Linder
1939	H. H. Whetzel	1940	E. A. Bessey
1940	D. H. Linder	1941	W. H. Snell
1941	E. A. Bessey	1942	J. N. Couch
1942	E. B. Mains	1943	F. D. Kern
1943	J. N. Couch	1944	N. E. Stevens
1944	G. W. Martin		

SECRETARY-TREASURER

1932–35	H. M. Fitzpatrick
1936–38	D. H. Linder
1939–41	J. N. Couch
1942–44	G. B. Cummins

COUNCILORS

1932	N. E. Stevens
1932–33	H. S. Jackson
1933–34	C. R. Orton
1934–35	L. O. Overholts
1935–36	C. L. Shear
1936–37	B. O. Dodge
1937–38	H. M. Fitzpatrick
1938–39	W. H. Weston
1939–40	L. O. Overholts
1940–41	H. H. Whetzel
1941–42	F. D. Kern
1942–43	D. H. Linder
1943	F. D. Heald
1943–44	C. W. Dodge
1943–44	E. B. Mains
1944–45	Lee Bonar
1944–45	J. N. Couch

EDITORIAL BOARD OF MYCOLOGIA

1933– F. J. Seaver, Managing Editor and Editor-in-Chief

1933	H. M. Fitzpatrick
1933–34	J. A. Stevenson
1933–35	F. A. Wolf
1933–36	G. R. Bisby
1933–37	E. B. Mains
1934–38	G. W. Martin
1935–39	J. A. Stevenson
1936–40	F. A. Wolf
1937–41	J. N. Couch (resigned Dec. 1939)
1940–41	F. K. Sparrow
1938–42	S. M. Zeller
1939–43	H. S. Jackson
1940–44	J. A. Stevenson
1941–45	J. H. Miller
1942–46	J. G. Hopkins
1943–47	A. H. Smith
1944–48	W. W. Ray

MEMBERSHIP COMMITTEE

F. J. Seaver, Chairman
S. M. Zeller
Erdman West
E. B. Mains

NOMENCLATURE COMMITTEE

D. P. Rogers, Chairman
H. M. Fitzpatrick
H. S. Jackson
E. B. Mains
F. J. Seaver
A. H. Smith
F. K. Sparrow

COMMITTEE ON MEDICAL MYCOLOGY

F. D. Weidman, Chairman
R. W. Benham
A. L. Carrion
N. F. Conant
C. W. Emmons
J. G. Hopkins
A. Howell, Jr.

REPRESENTATIVES ON COUNCIL A. A. A. S. (1944)

Chas. Thom — E. M. Gilbert

REPRESENTATIVE TO NATIONAL RESEARCH COUNCIL

N. E. Stevens

REPRESENTATIVES TO COUNCIL UNION OF AMERICAN BIOLOGICAL SOCIETIES (1944)

J. S. Karling — G. D. Darker

REPRESENTATIVE TO EDITORIAL COMMITTEE AMERICAN JOURNAL OF BOTANY

L. R. Hesler

FINANCIAL STATEMENT

December 31, 1942–December 31, 1943

Balance on hand December 31, 1942:		
Cash		$ 756.33
Government bonds		200.00
Savings account		600.00
Receipts:		
Annual dues in part 1943, 1944		1,659.67
Interest on savings account		11.31
Expenditures:		
New York Botanical Garden for Mycologia	$1,336.00	
Returned checks and discounts	11.39	
Postage and envelopes	24.69	
Secretarial help	15.75	
Mimeographing and printing	5.76	
Refunds to members	2.00	
Binding Society archives	9.15	
Bank service charges	4.46	
	$1,409.20	
Balance on hand, December 31, 1943:		
Cash	636.80	
Government bonds	940.00	
Savings account	241.31	
	$3,227.31	$3,227.31

(signed) GEORGE B. CUMMINS, *Secretary-Treasurer*

Examined and found correct:

(signed) H. C. GREEN, *Chairman of Auditing Committee*

Jan. 10, 1944

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[1] This index was prepared by Gussie Mildred Miller.

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